NEUROENDOCRINE STUDIES IN SCHIZOPHRENIA - AN APPROACH TO PATHOPHYSIOLOGICAL MECHANISMS

Submitted to the University of Glasgow for the degree of Doctor of Medicine

IAN NICOL FERRIER

Clinical Research Centre,
Harrow, HA1 3UJ

January, 1983

INDEX

List of Tables and Figures — Page No

ACKNOWLEDGEMENTS

My thanks are due to Dr. T.J. Crow for suggesting this project and for much encouragement and advice throughout. I am especially grateful to Dr. Eve Johnstone for her tireless help at many different levels and specifically for her help with clinical ratings. Dr. Mary Cotes provided detailed help and advice with RIA procedures and I must thank her for allowing so many valuable reagents to be put at my disposal.

Many colleagues have been helpful in several ways but I must specifically thank Drs. D.G.C. Owens, J.F. McMillan and P. McKenna for clinical assistance. Practical advice and help has also been readily given by Drs. A.J. Cross and C.D. Frith, for which I am most grateful. I would also like to thank Mr. S. Gamble, Miss S. Copping, Ms R. Bourne and Miss C. Canning for their technical assistance (documented in section on declaration of work done by author - page 257).

I would like to thank the consultant staff of Northwick Park and Shenley Hospitals for permission to study their cases. Thanks are also due to the nursing and paramedical staffs of these hospitals, notably the staff of Eastlake Ward, CRC.

The generous provision of RIA reagents and methods of Professor S.L. Jeffcoate, Professor W.R. Butt, Drs. W. Hunter, S. Sufi and P. Byfied is gratefully acknowledged. The assistance of the National Institute of Biological Standards, the US National Pituitary Agency and the WHO was invaluable.

I should like to acknowledge my gratitude to the MRC and to the Wellcome Trust for their financial support and trust!

Finally I should like to thank anyone I have forgotten, Mrs. G. Pain and Miss A. Kapoor for their typing and my father and my wife for their patience.

SUMMARY

Schizophrenia is a chronic and disabling disorder with marked resource implications for health care services. The nature of the underlying disease process(es) remains obscure. The hypotheses that a neurochemical abnormality underlies some of the manifestations of schizophrenia is plausible: the most likely candidate is an increase in dopaminergic neurotransmission. There are also indications of organic changes in schizophrenic brain which may underlie some of the features of chronic schizophrenia. The present series of studies investigate these putative pathophysiological mechanisms by examining the secretion of anterior pituitary hormones in unmedicated patients with accurately diagnosed acute and chronic schizophrenia.

The reasons for employing a neuroendocrine strategy are threefold. Firstly there is good evidence that hypothalamic neurotransmitters play a crucial role in the modulation of pituitary secretion: changes in these hormones may thus provide information on central neurotransmitter function. Neuroendocrine changes have been found in several neuropsychiatric diseases which are of potential clinical relevance. Finally there are several indications that a degree of hypogonadism is present in schizophrenic patients (pathological changes in the gonads reduced fertility, menstrual disorders and reductions in gonadotrophin secretion).

Several neuroendocrine studies are reported in this thesis. Firstly the basal levels of several pituitary, thyroid and gonadal hormones have been investigated in acute and chronic

schizophrenics and compared with controls. The rhythms of gonadotrophin, prolactin and growth hormone secretion have also been investigated (together with examination of the reproducibility of some of these measures). The hormonal response to synthetic hypothalamic releasing hormones has been studied in patients with chronic schizophrenia. In view of the interest in dopamine neurotransmission in schizophrenia the clinical and hormonal effects of dopamine antagonists and agonists in acute and chronic schizophrenics have been investigated. Pineal function in schizophrenia has also been examined and a paper discussing this bound into the thesis. Clinical associations have been examined and are emphasised throughout.

The results demonstrate selective reductions in gonadotrophin secretion in a subgroup of chronic schizophrenics which are associated with a reduction in the frequency but not amplitude of LH secretory episodes. While this pattern of abnormal secretion appeared to be reproducible within individual patients, the administration of acute or chronic dopamine antagonists was associated with a return towards normal levels. This suggests that dopamine overactivity may be implicated in the genesis of these abnormalities and this contention is supported by the finding that gonadotrophin abnormalities were associated with low prolactin secretion. These patients exhibiting these abnormalities had the longest length of illness and the more frequent positive symptoms of schizophrenia (e.g. delusions and hallucinations). The growth hormone response to a dopamine agonist was reduced in chronic schizophrenic patients as a group

and markedly so in a subgroup of patients, particularly those with negative symptoms which are characteristic of chronic schizophrenia and which are thought to have an organic basis.

By contrast basal levels and rhythms of putitary hormones were normal in acute schizophrenia. Nevertheless several important relationships were established in this group e.g. between prolactin secretion and 1) the positive symptoms of schizophrenia and 2) the dose and blood level of DA antagonists. These findings indicate that abnormalities of gonadotrophin secretion are unlikely to be of aetiopathological significance in schizophrenia but point to important relations between dopamine neurotransmission, clinical symptoms and anterior pituitary hormone secretion.

In addition, several clinical observations of the effects of dopamine agonists on schizophrenic patients were made which, although effectively negative results, have important theoretical implications for schizophrenia research.

The specificity, selectivity and significance of these results to the study of schizophrenia is extensively discussed in the text and possible areas of clinical relevance highlighted. The results strengthen the notion that there may be separate pathophysiological processes underlying different aspects of the schizophrenia syndrome and indicate that these hormonal changes may be useful markers of these processes. There are strong indications that there are abnormalities of peptide/dopamine interaction in the hypothalamus in schizophrenia. Some lines of future enquiry, based on these researches, are discussed which may help elucidate this complex and disabling disease.

1. INTRODUCTION:- Schizophrenia

Schizophrenia is a common and disabling disorder of unknown aetiology. It is characterised by disorders of thought, perception, emotion and motor behaviour. The estimated incidence of schizophrenia, 8 per 1,000 of the population, appears to be relatively constant in different countries, ethnic groups and social classes. Although some important advances in therapy have been made, schizophrenia is a disease associated with marked resource implications. It has been estimated that about 1/3rd of hospital beds in the United Kingdom are occupied by schizophrenic patients and that in the United States about 2% of the G.N.P. is spent on the care of schizophrenic patients. Despite considerable research the nature of the underlying disease process(es) remains obscure.

The studies reported here mainly concern the levels of pituitary and gonadal hormones in schizophrenic patients of differing clinical types before and after various pharmacological agents. The reasons for undertaking such studies are set out in detail below (Chapters 1.2.5 and 2.2) but to put these studies in context a description of the definitions, clinical course and outcome of schizophrenia, together with an evaluation of current aetiological theories, is first given.

1.1 The Nature of Schizophrenia

1.1.1 Definition of the syndrome of schizophrenia

The term schizophrenia was introduced by E. Bleuler (1902)

to denote those disturbances of psychological function which Kraepelin (1919) had previously grouped together under the heading of dementia praecox. Kraepelin's criteria for distinguishing dementia praecox from other psychiatric disorders was firstly the absence of marked disturbance of consciousness in dementia praecox and secondly that the outcome of dementia praecox was poor. Kraepelin stated that sufferers from dementia praecox seldom recover completely and that progressive deterioration of the personality occurs. Although Kraepelin himself recognized that these distinctions were no more than generalizations, they remain a cornerstone of clinical practice to this day. While it is apparent that it is unsatisfactory to define a disease by the exclusion of other diseases or retrospectively in terms of its outcome, subsequent attempts to define schizophrenia by the presence of features or symptoms which are characteristic of the disease have proved largely unsuccessful.

Bleuler (1911) formulated a series of 'primary' or 'fundamental' symptoms which he held to be characteristic of schizophrenia. These symptoms included a disturbance of associations between ideas, specific changes in affective response and ambivalence. However these symptoms are vague and it has proved difficult to devise operational definitions which can be applied to individual cases.

A more direct approach to the definition of schizophrenia was proposed by Schneider (1959) who suggested that the presence of certain symptoms - the so-called first-rank symptoms - defines an illness as schizophrenic. These symptoms include disorders

of the possession of thought, various forms of auditory hallucinations and delusional perceptions. Those symptoms have been incorporated, with some modifications, into the standardised diagnostic system of the Present State Examination (PSE) (Wing et al, 1974). It has been demonstrated that Schneider's symptoms, unlike Bleuler's, can be reliably assessed by independant observers (Wing and Nixon, 1975) but it has also been shown that these symptoms can occur, albeit with low frequency, in other psychiatric syndromes e.g. mania and confusional states (Wing and Nixon, 1975). Another problem with the phenomonological definition of schizophrenia incorporating Schneider's first rank symptoms is that it is not as good a predictor of poor outcome as other diagnostic systems which apply operational definitions (Kendall et al, 1978).

From the point of view of biological studies into schizophrenia there is no alternative to specifying the particular diagnostic criteria applied and also attempting to select criteria which are both reliable and valid (Crow et al, 1979). Where possible different systems of diagnosis should be employed so that an attempt can be made to identify biological correlates of specific psychopathological features and so that comparisons can be made between studies.

In the present study two systems of definition of schizophrenia have been used. Firstly the operational definitions of Feighner et al (1972) (which set out clearly defined criteria to be fulfilled) have been applied to the hospital casenotes. To further define the diagnosis the inventory of the P.S.E. (Wing

et al, 1974) was also applied to casenotes and the full interview of the P.S.E. was carried out where feasible. It is important to recognize that these two methods will identify separate but overlapping groups of patients. Further comments on the reliability and limitations of these methods are given in the subsequent relevant sections.

1.1.2 Clinical features of schizophrenia

While it is clear from the above account that there are no specific symptoms diagnostic of schizophrenia, the main symptoms can be classified as disorders of thought, perception, emotion and motor behaviour. Disorders of thought include disorders of the form, the stream, the possession and the content of thought. Disorders of form and stream of thought are seen clinically in the disturbed speech and vocabulary characteristic of schizophrenia known as formal thought disorder. Disorder of the possession and content of thought are seen clinically in the form of delusions. Delusions can be defined as false unshakeable beliefs which are out of keeping with the patient's cultural and educational background.

Disorders of perception in schizophrenia are seen in the form of hallucinations. Hallucinations are perceptions in the absence of external stimuli and, in schizophrenia, can be referable to all sensory modalities. All varieties of emotional disorder can occur in schizophrenia (for example anxiety and depression) but the abnormalities which are characteristically seen are flattening and incongruity of affect. Motor disorders are similarly varied and range from complicated stereotyped

behaviour patterns to abnormal movements of lips and tongue.

1.1.3 Course of schizophrenia

The onset of illness is very variable ranging from an acute illness fully developing over a few days to a slow insidious progression such that the exact point of onset is uncertain. Hospitalisation policy is variable and is therefore not a good determinant of onset. The natural history of the disease thereafter is similarly varied. Partly this depends on the criteria for diagnosis used as some systems have a degree of chronicity as a validating criterion. While some patients with a first episode have no subsequent morbidity, the normal course of schizophrenia is for remissions and relapses usually in the context of a degree of progressive deterioration.

1.1.4 Outcome

Kraepelin (1919) expressed the opinion that intellectual functions (e.g. orientation, consciousness and memory) were unimpaired in schizophrenia a view which was shared by Bleuler (1911). However Kraepelin did qualify his comments to describe 'a terminal condition of dementia' in schizophrenic patients who exhibited 'a general decay in mental efficacy' and who become 'impoverished in thought, monotonous in mental activity' (Kraepelin, 1919).

Since Kraepelin described these terminal states it has become recognized that a proportion of schizophrenic patients proceed to a state characterized by lack of productive activity, social withdrawal and occasionally mutism (the "defect state").

It has become recognized that many patients with chronic schizophrenia perform poorly on tests of intellectual function. Some observations suggest that these impairments are due to reduced volition and that improvements are seen if the conditions of institutional life are made more interesting. However there is evidence that similar impairments are found in non-institutionalized chronic schizophrenics (Johnstone et al, 1981). It has also been shown that there is a relationship between the characteristic symptoms of the defect state (the negative symptoms - flattening of affect and poverty of speech) and cognitive and neurological impairments of chronic schizophrenic patients (Owens and Johnstone, 1980) which suggests an organic basis to the defect state. These observations have prompted a series of investigations into the disabilities and defects of these patients with chronic schizophrenia some of which will be reviewed below (see Section 1.3.4).

1.2 Aetiological theories of schizophrenia

1.2.1 The genetic component

There is no doubt that the incidence of schizophrenia is much higher among the relatives of schizophrenics than in the general population. The most persuasive evidence for a genetic determination comes from concordance studies of mono- and dy-zygotic twin pairs. Shields (1976) reported that the concordance rate of schizophrenia for mono-zygotic twins lies between 45 and 65 percent. By contrast, the concordance rate in same-sex dyzygotic twins lies between 12 and 14 percent. That these

rates are due to a genetic component rather than environmental factors is demonstrated by the fact that similar rates of concordance are observed in studies of monozygotic twins reared apart. The genetic contribution is further supported by studies which demonstrate that the incidence of schizophrenia in adopted children resembles that of their biological parents more closely than that of their adoptive parents. Although the evidence for a genetic component is persuasive, the fact that the concordance in monozygotic twins is less than 100% indicates that other factors are also relevant. Moreover it is clear that the mode of inheritance within families is not readily compatible with any one simple theory of genetic transmission (Shields, 1976). Thus schizophrenia may be analogous to other chronic diseases such as rheumatoid arthritis, in which there is a genetic predisposition and the disease is precipitated by various environmental factors.

1.2.2 Personality, environmental and family studies

The role of the personality in the causation of schizophrenia has been the subject of discussion for a long time. No clear cut predisposing personality type or environmental factor has been identified. Such studies are fraught with problems and methodological difficulties. For example the personality and relationship difficulties of the schizophrenic patient are always viewed retrospectively by investigators only after the diagnosis is made and the question of similar problems applying to the non-schizophrenic population is rarely

tackled. It does seem that non-specific environmental changes ('stress') may precipitate schizophrenic illness (Birley and Brown, 1970). However why some individuals and not others of the same family develop schizophrenia in this context is not clear. It may be that (in many cases), early schizophrenic illness itself leads to environmental changes.

1.2.3 Neurochemical hypotheses of schizophrenia

As discussed above, with certain reservation regarding the defect state, schizophrenia can be distinguished phenomenologically from the organic states on the basis of the absence of marked disturbance of consciousness in schizophrenia. This may throw some light on pathogenesis. As Bonhoeffer (1909) pointed out the psychotic reactions associated with physical disease as varied as infection, anaemia, thyroid disturbance etc. cannot be distinguished clinically. A similar arguement applies to chronic organic states, the dementias - illnesses which share with the acute organic psychoses disturbances of learning capacity and intellect. This suggests that in these organic psychoses there is a generalised disturbance in those cells concerned with cognitive activities and that this represents an insult on a significant fraction of the total cerebral mass. Since cognitive processes are generally unaffected schizophrenia must result from a cellular disturbance of a more discrete nature. Since the identity of the neurotransmitter is a functionally important feature which distinguishes systems of neurones, it may be the characteristic which distinguishes between cells affected and those not so affected.

Any neurohumeral hypothesis of schizophrenia is greatly strengthened if it can be shown that drugs which are known to alter neurotransmitter function in the CNS in a predictable way, have effects on characteristic schizophrenic symptoms in a way which is predicted by the hypothesis. The neurochemical hypotheses of schizophrenia fall into three main groups a) inborn errors of metabolism b) production of a toxin with psychotogenic properties c) neurotransmitter dysfunction. Table 1.1 outlines the main neurotransmitter hypothesis of schizophrenia with a description of supporting and confounding pharmacological and biochemical evidence.

Proposed inborn errors of metabolism are based solely on observations on the body fluids or brain tissue of schizophrenics and do not take into account any pharmacological evidence. These hypotheses have either failed to be confirmed or shown to be artefactual.

There is little evidence to support the hypotheses of production of an endogenous psychotogen or neurotoxin. Initial claims of increases in methylated amines, which resemble some known hallucinogens, have not been confirmed in subsequent studies. In any event, it is necessary to explain how such postulated toxins exert their psychotogenic effects and it is likely that some specific neuronal system, and therefore neurotransmitter, would be involved.

The first neurochemical hypothesis of schizophrenia was advanced by Wooley and Shaw (1954). These authors proposed that the symptoms of schizophrenia were attributable to failure of

TABLE 1.1

HYPOTHESIS	PROPOSERS/DATE	PHARMACOLOGICAL ARGUMENT AND EVIDENCE		POST-MORTEM BIOCHEMICAL EVIDENCE		REFERENCES
		FOR	AGAINST	FOR	AGAINST	
Serotonin deficiency	Wooley and Shaw (1954)	LSD psychosis resembles schizophrenia. LSD blocks serotonin receptors	but with some fundamental differences! Tryptophan and 5-hydroxy-tryptophan therapeutically ineffective	-	Serotonin turnover not decreased Serotonin receptors not altered	Owen et al, 1981
Noradrenaline neurone deficiency	Stein and Wise (1971)	Reward processes mediated by NA systems: Anhedonia a common symptom	Blockade of DBH by disulfiram does not precipitate schizophrenia	-	Dopamine-β-hydroxylase not altered. α and β receptors unchanged	Owen et al, 1981
GABA deficiency	Roberts (1972)	GABA inhibits monoamine neurones. Disinhibition of monoamine neurones consistent with schizophrenia	Baclofen and muscimol are therapeutically ineffective	-	GABA levels and receptors unaltered	Owen et al, 1981
Opiate excess	Bloom et al (1973)	Opiate induce catatonia. Naloxone antipsychotic in some circumstances	Conflicting evidence on clinical effects of opiate agonists and antagonists	-	Opiate receptors unaltered	Owen et al, 1978
Dopamine increase	Randrup and Munkvad (1965)	Amphetamine psychosis resembles schizophrenia. Amphetamines release dopamine	See text	Dopamine receptors increased (see text)	Dopamine turnover not increased (see text)	See text

Neurochemical hypotheses of schizophrenia

serotoninergic neurotransmission. This hypotheses was based on the fact that lysergic acid diethyamine (LSD) blocked some of the pharmacological effects of serotonin and so the psychotogenic effects of LSD were attributable to central serotonin receptor blockade. There is however little evidence to support this hypothesis (see Table 1.1). The clinical features of LSD psychoses differ significantly from those of acute schizophrenia and there is no supporting evidence from PM studies , CSF, blood or urine studies of schizophrenics (Table 1).

More recently the hypothesis has been advanced that a degeneration of central noradrenaline (NA)-containing neurones might account for the symptoms of the 'defect state' (Stein and Wise, 1971). This hypothesis rests upon observations on the behavioural effects of the neurotoxin 6-OH-dopamine, which destroys Na-containing neurones. There is little supporting evidence for this hypothesis (Table 1.1) and good evidence against it e.g. 3-methoxy-4-hydroxy-phenylethylene-glycol (MHPG) - (an indicator of CNS turnover of Na) is not abnormal in schizophrenia, inhibition of dopamine-β-hydroxylase (DBH) (an enzyme specifically associated with Na neurones) does not precipitate a schizophrenia-like illness and D-B-H itself is not abnormal in schizophrenic brain (see Table 1.1). Abnormalities of GABA transmission and opiate mechanisms have been postulated in schizophrenia. Neither of these hypotheses is supported by PM studies or neuropharmacological studies with schizophrenic patients. Details are given in Table 1.1.

The hypotheses, first put forward by Randrup and Munkvad (1966), that there is dopamine (DA) overactivity in schizophrenia

is however supported by some evidence. Firstly it is known that amphetamine intoxication or abuse can lead to a syndrome that is clinically indistinguishable from schizophrenia - a similarity that extends beyond florid, acute features to some similar affective changes (Connell, 1956). The pharmacological effects of amphetamine include the release of Na and DA from nerve terminals and blocking of re-uptake. The principal action of amphetamine when administered to humans appears to be release of DA. The schizophrenic-like psychosis of amphetamine abuse is rapidly and effectively reversed by DA receptor blocking drugs (neuroleptics) (Angrist et al, 1974) and not by α or β adrenergic receptor blockers. Animal work, which demonstrates a characteristic behavioural pattern with stereotypies and reduced social interactions following amphetamine administration (Ridley et al, 1979), also supports the contention that the main behavioural actions of amphetamine are DA mediated.

The second body of evidence in support of the Da overactivity hypothesis of schizophrenia is the therapeutic specificity of neuroleptics in schizophrenia. It has been shown in several large well designed placebo controlled studies (NIMH Study Group, 1964) that neuroleptics have a specific action in ameliorating typically schizophrenic features (e.g. delusions and hallucinations) and in preventing their recrudesence (Hogarty et al, 1974) - an action which is not secondary to their sedative properties. It has been shown that there is a strong relationship between the DA-blocking potency of neuroleptics and the therapeutic dose - a relationship which is not found with their

anticholinergic and α-adrenoreceptor blocking potency (Creese et al, 1976) - and that isomeric forms of neuroleptics with no DA blocking potency are not clinically effective (Johnstone et al, 1978).

Thus it would appear that excessive DA release can induce a schizophrenic-like psychoses and that DA blockade can relieve schizophrenic symptoms. These observations lead to the study of DA receptors in schizophrenic brain. It has now been shown by several groups of workers that there is an increase in the numbers of DA receptors in the striatum (putamen, caudate and nucleus accumbens) of post mortem schizophrenics brain (Owen et al, 1978; Lee et al, 1978); DA metabolism in the pre-synaptic neurones (as judged by the ratio of DA to homovanillic acid (HVA)) is however unaffected (Owen et al, 1978). The main problem with these studies is that many of the patients had been treated with neuroleptics prior to death. It has been shown in animals that neuroleptic medication increases the numbers of striatal DA receptors (Muller and Seeman, 1978). There is however evidence that increases in DA receptors are found in some patients drug free for some time prior to death (Owen et al, 1981). Moreover the DA receptor increase in schizophrenic brain is confined to one particular type of DA receptor (the DA2 receptor - according to the classification of Kebabian and Calne, 1979) which is not elevated with chronic neuroleptic therapy in animals whereas the DA1 receptor which remains elevated for some time after neuroleptic withdrawal is unaltered (Cross et al, 1981).

There is thus some pharmacological evidence to support the

DA overactivity hypothesis of schizophrenia and a possibility that a pathophysiological basis underlying schizophrenia has been identified (increased post synaptic DA receptors). However some major weaknesses of the DA overactivity hypothesis have been revealed in the search for evidence of increased DA release or receptor supersensitivity in untreated schizophrenic patients. CSF studies of HVA suggest that DA turnover is not increased either in acute or chronic schizophrenia (Bowers et al, 1974). It has been established that schizophrenia and Parkinson's disease (in which there is DA depletion) can co-exist, and that one disease does not substantially modify the features of the other, which suggests that increased DA release is not necessary for schizophrenic symptoms (Crow et al, 1976). There are also some outstanding questions regarding the pharmacological evidence. It is uncertain whether neuroleptics act upon the entire range of symptoms of the disease - it seems probable that neuroleptics do not influence features of the defect state (for example the negative symptoms (affective flattening and poverty of speech) (Letemendia and Harris, 1967)) and amphetamine administration appears to have little effect on chronic schizophrenic patients (Kornetsky, 1976).

There are two additional problems for the DA overactivity hypothesis of schizophrenia which have direct relevance to the present studies. Firstly it is clear that there is considerable heterogenity among the schizophrenic population in terms of clinical response to neuroleptics, clinical outcome, CAT scan abnormalities (see Section 1.3.4) and DA receptor numbers in

post mortem studies. This raises the possibility of different pathophysiological processes underlying schizophrenia and emphasizes the need for measurable biological markers of disease. Secondly, prolactin (PRL) secretion from the anterior pituitary gland is normal in schizophrenia (Meltzer et al, 1974). It is well established that hypothalamic DA is inhibitory to PRL release and that PRL levels provide a sensitive index of DA blockade induced by neuroleptics. If the DA hypothesis is valid and if DA overactivity extends to the tubero-infundibular neurones (TIDA neurones see Section 2.1.2) then low PRL secretion would be anticipated in schizophrenia (Johnstone and Ferrier, 1980 for review).

Evaluation of the neurohumeral hypothesis. An attempt to integrate the diverse findings concerning possible neurochemical abnormalities in schizophrenia is bound to fail at the present time. Too many questions concerning, for example, the validity of techniques for assessing monoamine turnover or the validity, of otherwise, of clinical distinctions such as acute or chronic, paranoid or non-paranoid, schizophrenia remain. At the present time, the DA hypothesis has some strengths - notably its ability to explain the two major pharmacological facts about schizophrenia - that symptoms are ameliorated by neuroleptic drugs and mimicked or exacerbated by amphetamines - but some major weaknesses. There is an established need for an index of central events that could be applied to all clinical varieties of schizophrenia, particularly the defect state where evidence of organicity is accumulating (see below).

1.2.4 Disorders of the nervous system - pathological and structural changes in schizophrenic brain

For long it has been believed by many psychiatrists that schizophrenia is due to a disorder of the brain, but evidence for this has been a long time coming. There have been several isolated reports of schizophrenia in association with recognized CNS lesions (e.g. tumours etc) in diverse brain regions. Any association between cerebral disease and schizophrenics raises three questions: 1) whether the association occurs by co-incidence 2) whether the cerebral disease merely makes manifest a latent tendency to schizophrenia or 3) whether schizophrenics are more prone than the rest of the population to develop cerebral disease.

Extensive studies have revaled several associations between cerebral disease and schizophrenia. Davison and Bagley (1969) came to the conclusion that the association between schizophrenia and cerebral tumour and injury was significantly greater than chance and that the association was with lesions in the temporal lobe. Slater et al (1962) found that the mental disturbances associated with epilepsy had many similarities with schizophrenia. Several studies have indicated that it is damage to the limbic structures in the temporal lobe which link temporal lobe epilepsy and schizophrenia (Torrey and Peterson, 1974). Birth complications and neonatal injuries which are known to damage the temporal lobe are found with increased normal frequency in schizophrenic patients. There are several circumstantial lines of evidence which raise the possibility of a viral aetiology of

schizophrenia and some evidence of schizophrenia-like psychoses in patients with temporal lobe encephalitis (Torrey and Petersen, 1974). A virus like effect of schizophrenia CSF on cell culture has recently been demonstrated but subsequently found to be present in a variety of neurological illnesses (Crow et al, 1979). These studies are all suggestive of a pathological process in the brain, perhaps in the temporal lobe, in schizophrenia. However several neuropathological studies have failed to demonstrate evidence of a consistent pathological change in schizophrenic brain. Some of these earlier studies lack properly designed control groups. More recent studies, which have examined controls, have only demonstrated patchy gliosis and reduced cell counts in some areas in some cases (Dom, 1976; Stevens, 1981).

The development of radiological proceedures over the last twenty years has however produced a number of consistent observations which have renewed interest in the search for a neuropathological basis of schizophrenia. Following the introdunction of pneumoencephalography, there have been a number of reports that schizophrenics have evidence of cerebral atrophy and enlarged ventricles. Some of the earlier studies lacked proper assessments and controls, but a summary of subsequent studies where an attempt has been made to overcome these difficulties is given in Table 1.2. Table 1.2 also includes details of some of the many recent studies on schizophrenic patients using computerised axial tomography (CAT scans).

It has become clear that schizophrenics as a group have

TABLE 1.2

Study	Date	Neuroradiological Procedure	Patients Studied	Main findings
Asano	1967	Pneumo-encephalography	53 Schizophrenics	Dilation of lat. and 3rd ventricles in 70% of cases
Holden et al	1973	Echoencephalogram	79 Schizophrenics 79 Controls	Dilatation of 3rd ventricle associated with treatment resistance
Johnstone et al	1976	CAT	17 Chronic Schizophrenics compared with controls (matched age and premorbid occupation)	Ventricle size larger in patients than controls ($p < 0.01$)
Weinberger et al	1979	CAT	65 Schizophrenics 56 Controls (age matched)	Dilatation of lat. ventricles in 50% of cases associated with poor treatment response
Takahashi et al	1981	CAT	169 Schizophrenics	Dilatation of 3rd and lat.ventricles associated with "blunted affect".

Examples of studies investigating ventricle size in schizophrenia. Dilitation seen by each of the three different neuroradiological methods.

enlarged ventricles although there is an overlap with the normal elderly population and with other diagnostic groups. Ventricular dilatation has been demonstrated in some young patients soon after the initial diagnosis, but in general the abnormality(ies) are found in patients who have been ill for many years. Table 1.2 also indicates some of the clinical associations of ventricular dilatation that have emerged in these various studies. Overall there is an association between ventricular dilatation and intellectual impairment, poor pre-morbid adjustment, resistance to therapy, poor outcome and perhaps negative symptoms - features which correspond with the defect state. There is no evidence, surveying these papers together, that there is a relationship between ventricular dilatation and florid ('positive') symptoms of schizophrenia such as delusions and hallucinations. Thus it appears there is good evidence that a subgroup of schizophrenic patients have structural brain disease. The size of this subgroup, the clinical significance of the cerebral changes and the fundamental cause of them however remains to be determined.

Table 1.2 also indicates those studies were the size of the 3rd ventricle has been measured. Three studies, each using a different technique, have demonstrated enlargement of this ventricle. The patients in whom this was found fall into the same category as those described above i.e. those with evidence of deterioration. The hypothalamus surrounds the third ventricle and is a principle endocrine and autonomic control centre. The possible relevance of dilatation of the 3rd ventricle in

schizophrenia and endocrine abnormalities in schizophrenia is discussed below.

1.2.5 Endocrine disorders in and their relationship with schizophrenia

The idea that mental disorders and endocrine diseases may be related has its origins in antiquity. In more recent times Kraepelin (1919) postulated that dementia praecox was basically an endocrine disorder and Freud observed that disorders, which he was seeking to understand by psychological means, would eventually be treated with hormones. The reasons behind this hypothesis are that 1) schizophrenia is occasionally associated with endocrine events (e.g. puberty, the puerperium, menstruation and the menopause) and 2) schizophrenia or schizophrenic-like illnesses are occasionally seen in patients with established endocrine disease (e.g. Cushing's syndrome, hypothyroidism etc.).

A critical review of these associations however leads to a less positive conclusion than that of Kraepelin cited above. Although the onset schizophrenia is very rare prior to puberty, several well documented cases have been described. A few such cases were seen in the population from which the patients studied here were drawn (Owens and Johnstone, 1980). Furthermore there is no known relationship between the age of onset of puberty and the age of onset of schizophrenia.

Schizophrenia occasionally develops in the puerperal period. The clinical distinction between puerperal schizophrenia and other psychotic illneses developing in this period is however often problematical. The association between the development

of schizophrenia and the puerperium may be casual rather than causal in some cases and the relationship of puerperal schizophrenia to non-puerperal schizophrenia in terms of prognosis remains to be clarified.

While changes in mood associated with menstruation and the menopause occur in many females, reports of an increased incidence of schizophrenia at these times are unreliable. Periodic psychoses associated with menstruation has been described (Endo et al, 1978) but an examination of the clinical data does not support the contention that these psychiatric episodes are schizophrenic. There is no direct epidemiological evidence to link the menopause or disorders of the menopause with schizophrenia.

While there is no doubt that psychological symptoms such as apathy, irritability and anxiety are common in established endocrine disease (e.g. Addison's disease, thyrotoxicosis), the incidence of psychotic illness is much less. The illnesses in which psychoses are most commonly seen are Cushings syndrome where the incidence is about 20% and hypothyroidism where the incidence in severe cases is about 10%. Treatment with high doses of steroids leads to a psychotic illness in about 5% of cases (Michael and Gibbons, 1963). Although the symptoms of these psychotic episodes are occasionally typically schizophrenic (catatonia and first rank symptoms have been reported) more usually the psychoses have a strong affective and/or confusional component. Treatment (with adrenalectomy and thyroxine respectively) is almost invariably curative.

The observations of the clinical associations of schizophrenia with steroid excess and thyroid hormone deficiency prompted a series of investigations into the levels of these hormones in schizophrenia. Reports of steroid excess or deficiency in schizophrenia on indirect measures were made originally but direct measurements of plasma corticosteroids revealed no consistent abnormalities (Michael and Gibbons, 1963) particularly in "chronic affectless schizophrenics" (Bliss et al, 1955). Similarly uptake of radioiodine, protein bound iodine (Michael and Gibbons, 1963) and thyroxine (Kline et al, 1968) have all consistently been found to be in the normal range in acute and chronic schizophrenics.

Thus the investigation of the many clinical associations between schizophrenia and endocrine disease has failed to provide an adequate pathophysiological basis for schizophrenia. However these associations are still of great theoretical interest and further investigation of cases of endocrine disorder in which schizophrenia develops may yet throw light on the aetiology of schizophrenia in general. One possibility raised by these clinical associations is that schizophrenia is a common end point of various pathological processes. This idea will be discussed further in chapter 8.

Another aetiological hypothesis of schizophrenia that has been put forward is that disorders of the hormones or the organs involved in reproduction may be responsible for schizophrenia. This hypothesis will now be examined in depth. The evidence for an abnormality of gonadal function is first presented followed

by a critical analysis of this hypotheses.

1. Pathological studies

Kraepelin (1919) first postulated that dementia praecox (schizophrenia) realted to disease of the endocrine glands, especially the gonads. He suggested that toxins from the sex glands were the probable cause of the mental disease. This idea was taken up by Sir F. Mott (1919) who carried out a large series of post-mortems at the Maudsley Hospital on cases of dementia praecox. He described profound degeneration and fibrous change in the gonads of these patients. He found that the longer the illness the greater the degree of degeneration, although he also described profound changes in material from patients with acute illnesses. He was adamant that chronic diseases (such as tuberculosis) were not the cause of the findings. Mott held that these changes were a result of suppression of the libido in adolescence.

These observations were partially confirmed in subsequent studies. Lewis and Davis (1921) studied 140 cases of dementia praecox and 450 controls and concluded that gonadal atrophy was much more common in schizophrenia. Morse (1923) agreed with this conclusion but felt that the frequency of gonadal pathology had been overestimated. He also felt that there was no relationship between the pathological findings and "psychic state". McCartney (1929) reported fibrosis, degeneration and sclerosis of the interstitial components of the tests in post-mortems from 158 schizophrenics but reported that spermatogenis was not significantly altered from that found in 260 controls. McCartney

felt that he had accrued enough evidence to state that "dementia praecox is primarily an endocrinopathy in which the gonads are consistently degenerated and hypo-functioning". McCartney found that toxaemias of various types, notably tuberculosis, were more common in the schizophrenic population but felt that this was not the cause of the high frequency of pathological changes in the testes of the schizophrenics. Pathological abnormalities were found by Hemphill et al (1944) in a study of testicular biopsies from 90 schizophrenics. In distinction to the findings of McCartney (1929) but not those of Mott (1919) they found degeneration of the semeniferous tubicles and a failure of spermatogenesis but no abnormalities of the interstitial components of the testes.

The most recent pathological study of the testes in schizophrenia was performed by Blair et al (1952) who examined testicular biopsies from 14 schizophrenic patients. No consistent pathological abnormality was detected in any of these patients. These authors attributed all the findings of the previous studies to the effect of the high frequency of diseases such as tuberculosis in schizophrenic subjects and to the effects of the poor diet of inmates of mental hospitals. As has been pointed out above these possibilities were considered by earlier authors and refuted by studying controls from the same institution as the schizophrenics and by looking specifically for the (then well recognized) changes of tuberculosis. Another problem with the Blair et al (1952) study (which is now considered as the definitive study) is that the patients had been ill for a

relatively short time (3 - 7 yrs) while the previous studies had indicated that abnormalities were particularly found in patients who had been ill for many years.

Thus there does appear to be at least some evidence for pathological abnormalities in the gonads of schizophrenics and an indication that the pathophysiological basis for these abnormalities should be sought. These pathological studies are particularly relevant in view of the fact that all of them antedate widespread neuroleptic drug therapy for schizophrenia.

2. Studies of the fertility of schizophrenics

It is well recognized that the fertility of schizophrenics as a group is reduced (Stevens, 1969). Most investigators do not consider the possibility that this may be accounted for by a pathological process and search for the underlying sociological and psychological reasons. It is established that schizophrenics marry less frequently, that many schizophrenics have difficulty with psychosexual adaptation, that regular sexual intercouse with a partner of the opposite sex is often not established, that the rate of marriage breakdown where one or both partners are schizophrenics is high and that there is frequently physical separation due to hospital admission. In addition patients with schizophrenia frequently experience such problems as anxiety, depression, social uncertainity, withdrawal, apathy and not infrequently, sexual delusions which hinder the establishment of a normal sexlife.

Thus it is not difficult to see why investigators have concentrated on sociological and psychological reasons for the

reduced fertility of schizophrenia. Nevertheless since fertility is also reduced when a schizophrenic patient's marriage is well established (Stevens, 1969) it is plausible that reduced fertility is in part due to inadequate sperm or ova production in schizophrenia. Unfortunately there is no reliable evidence to answer this question at present (see next section).

3. Studies of sexual function and development in schizophrenia

a) Males. There are considerable difficulties in assessing degrees of sexual dysfunction which are even more profound when studying schizophrenic patients. It is not known whether abnormalities of puberty, libido, potency or ejaculation occur in male schizophrenics. One study of sperm production has been made in schizophrenia with conflicting results (Kline et al, 1968) and the normality or otherwise of spermatogenesis from pathological studies is disputed (see above).

It appears that the development of secondary male sexual characteristics such as scrotal development, beard hair, voice change is within normal limits in schizophrenia. Part of normal male sexual development is the development of the male body habitus. This is characterised by the development of broad shoulders and narrow hips. An index of male physique can be made by measuring the biacromial and bi-iliac diameter. Using this index - the discriminant-androgyny score (Tanner, 1951) - several groups of workers have reported significantly lower, that is more feminine, scores in groups of patients suffering from schizophrenia - although the frequency of abnormality varies

from about 75% (Rey and Coppen, 1959) to 20% (Brooksbank et al, 1970) of the patients studied. Thus there is at least some evidence to suggest failure of secondary sexual development in schizophrenic patients.

b) Females. The general comments regarding the difficulties of assessing sexual function in male schizophrenic patients apply equally to female schizophrenic patients. It is well recognized that menstrual disorders are common in schizophrenic patients. The exact frequency of menstrual disorders in schizophrenia is unknown and the relationship of these disorders to schizophrenic symptoms, relapses and remissions and hospitalisation and to the reduced fertility of schizophrenia is also uncertain. In a series of studies Gimes et al (1969) and Ripley et al (1941) examined cervical and vaginal smears from a large number of female psychiatric patients and demonstrated that in schizophrenia the frequency of amenorrhoea or oligomenorrhoea was 75% and that in these subjects there was a hypofollicular cytological picture compatible with ovarian dysfunction and failure of ovulation. In general, both these groups of workers found that the more severe the psychoses the more marked the evidence of ovulatory failure. In some cases there was a reversal of the abnormal cytological pattern on clinical remission though the statistical basis of this observation is open to doubt. Gimes et al (1969) felt that they had uncovered clear evidence of "disturbed hypothalamic neural regulation" in schizophrenia.

However the precise significance of these findings is

uncertain. It is anecdotally and scientifically recognized that menstrual irregularities are common following many situations (for example life events, severe stresses, neuroses and depression). There is convincing evidence of stress related hypothalamic dysfunction (Quigley et al, 1980). Thus it is unclear to what extent evidence of menstrual disorders, ovulatory failure and possible hypothalamic dysfunction in schizophrenia represents a non-specific reaction to psychic phenomena, changes in environment etc and to what extent they may be a pathophysiological consequence of the disease process of schizophrenia.

4. Hormonal studies in schizophrenia

Over the last thirty years there has been a large increase in knowledge about normal endocrinological control. The development of radio-immunoassay (RIA) (Berson and Yallow, 1967) in the late sixties has lead to sensitive and specific assays for the hormones involved in reproduction. Further technical developments have lead to the elucidation of the complex factors involved in the process of reproduction. This topic will be fully developed in the next chapter. In this section the argument that hormonal imbalance may be an aetiological factor in the development of schizophrenia is continued and an outline of the outstanding reproductive hormonal abnormalities in schizophrenia is given below.

Hoskins and Pincus (1949) investigated the secretion of the gonadol steroids, androgens, oestrogens and of the 17 keto-steroids, in the urine and blood of 29 schizophrenic men as part of a series of endocrine investigations in schizophrenia

carried out at the Worcester State Mental Hospital, Massachusettes, USA. They found that the schizophrenic men differed from normal controls in three measures:- a) an elevated total oestrogen output, b) a lower androgen output and c) a lower androgen/oestrogen ratio which tended towards female levels. They also reported that there was a subgroup of patients with markedly reduced androgen/oestrogen : these patients were said to be much less aggressive than the remainder of the patients. Similar findings were reported by Sands (1957). Although clinical information is scanty in his essentially descriptive study, he appeared to find reduced androsterone secretion in schizophrenia and a very variable pattern of 17-oxosteroid secretion. In 1964 Brooksbank and Pryse-Phillips reported that the mean excretion rate of 3 α-hydroxy-androst-16-ene in 33 schizophrenic patients was markedly lower than that of healthy men and other psychiatric patients. However in a subsequent study Brooksbank et al (1970) were unable to confirm this finding although they did demonstrate a subgroup of the patients with extremely low levels of this hormone and low levels of urinary testosterone.

With the development of increasing knowledge of normal endocrine control attention has moved from gonadal steroid secretion to pituitary hormone secretion in schizophrenia. The first such investigation was carried out by Shader et al (1968). These workers measured the urinary secretion of follicle-stimulating hormone (FSH) by a bioassay in 10 male unmedicated chronic schizophrenics. There was a marked reduction in urinary FSH in 4 of these patients and it was found that these patients

had been ill for longer than those with normal FSH secretion and that they also had a younger age of onset of schizophrenia. In addition FSH levels were significantly related to the mental state of the patients: urinary FSH was negatively associated with increased psychopathology.

The development of RIA made the assessment of pituitary hormone secretion much more direct. In 1974 Dr. Brambilla and her collaborators reported that the secretion of FSH, luteinizing hormone (LH) and testosterone were reduced in a group of 20 schizophrenic patients compared to age-matched controls. The patients were described as suffering from chronic hebephrenic schizophrenia of 'puberty onset'. The study was performed after neuroleptic therapy had been withdrawn. The reductions in FSH, LH and testosterone were marked (50%, 20% and 16% of control values respectively) and each hormone was significantly reduced at the $p < 0.001$ level. The hormonal pattern reported did not conform to any recognized endocrine disorder and suggested a hypothalamic-pituitary deficiency in schizophrenia.

This study was followed comprehensively by Dr. Brambilla in three separate but overlapping studies:

1) Brambilla et al, 1975. This investigation concerned the effects of neuroleptics on the reduced secretion of LH, FSH and testosterone in schizophrenic patients. They reported that the re-introduction of neuroleptic therapy (haloperidol 6 mg/day i.m.) was associated with the return of all three hormones to the normal range. This result is important for two reasons:-

a) it is evidence against prior administration of neuroleptics

as the cause of the original findings and b) it raises the possibility of a connection between schizophrenia, dopaminergic neurotransmission in hypothalamic-pituitary axis and hormonal abnormalities.

2) Brambilla et al (1976). In this paper it was reported that the FSH and LH responses to synthetic luteinizing hormone releasing hormone (LHRH) were enhanced compared to control values. This response was held to be similar to that observed by Roth et al (1972) in children undergoing sexual maturation and to patients with the sexual immaturity syndrome. This pattern of hormonal response to LHRH in chronic schizophrenics was different from that found in patients with hypogonadotrophic hypogonadism (Roth et al, 1972) (also known as idiopathic gonadotrophin deficiency : IGD) and this distinction was amplified in:-

3) Brambilla et al (1977). This paper reported the results of the administration of clomiphene citrate (a non-steroidal oestrogen analogue which blocks oestrogen and androgen-sensitive receptors in the hypothalamus and abolishes their inhibition of gonadotrophin release) to a group of chronic schizophrenic patients. The patients showed a normal increase of FSH level during clomiphene administration but with markedly blunting of the LH and testosterone response. This pattern of hormonal response to clomiphene is also different from that of IGD patients and resembles more closely those patients with defects in spermatogenisis.

In conclusion these primary papers of Brambilla and co-workers indicate a) reduced reproductive hormonal secretion in

chronic schizophrenia b) which can be reversed by neuroleptic drugs and c) that FSH and LH respond to dynamic tests of their secretion in different ways and d) that there are important differences between the hypogonadism of schizophrenia and that of IGD patients. There are two main objections to these studies:- 1) the patients studied were all of one unusual clinical type (chronic hebephrenic schizophrenic patients with a very early age of onset) and 2) the patients when studied had only recently (1 - 3 weeks) been withdrawn from neuroleptic drugs. These objections lead to a study by Johnstone et al (1977) who reported on LH and FSH secretion in a group of chronic schizophrenic patients who had never received neuroleptic drugs. This study confirmed the finding of low LH and FSH secretion in chronic schizophrenics although it was found that abnormalities were only detected in a proportion of the patients. Interestingly, this work confirmed Shader et al's (1968) observation of decreasing FSH with increasing schizophrenic symptomatology and found a significant inverse relationship between delusions and FSH secretion.

Evaluation of studies on a hormonal aetiology of schizophrenia

As discussed at the beginning of the previous section there are some clinical observations which indicate a relationship between hormonal events (e.g. puberty, puerperium, menstruation) and schizophrenia. It is now accepted that there is little evidence that abnormalities of thyroid function or adrenal secretion are present in schizophrenia or are in any way related to the schizophrenic process. There is however evidence that abnormalities of the hypothalamic-pituitary-gonadal

axis are present in schizophrenia which can be summarised as follows:-

1) pathological abnormalities in the gonads have been detected by several groups of workers.

2) fertility is reduced in schizophrenia to an extent which probably cannot be completely accounted for by sociological causes.

3) there is some evidence of incomplete and inadequate sexual development and function in schizophrenia and

4) evidence of disordered pituitary-gonadal secretion in schizophrenia has been reported.

There are however two main drawbacks to the hypothesis that an abnormality of reproductive hormones is an aetiological factor in schizophrenia. Firstly schizophrenic symptoms are not found with any regularity in any of the well-recognized conditions associated with reproductive hormone abnormalities. It is known that schizophrenic symptoms are not increased in frequency in conditions such as testicular or ovarian failure, gonadal dysgenesis, hypopituitorism etc. Furthermore there is no evidence of schizophrenic features in Kalman's syndrome which is characterised by congenital failure of gonadotrophin secretion. The only dissenting voice to the above statements is that of McCartney (1928) who described a "typical schizophrenic affect" in 24 male eunchs who had been castrated on the orders of the Emperor of China. Since these individuals were also subjugated to the Imperial Court of China in the feudal times of China in 1928 and since the actual symptoms described appear mild and

non-specific it would appear that the symptoms described could be accounted for by the "environmental viscissitudes" of these subjects lives (Hoskins, 1943).

Thus it appears unlikely that gonadotrophin and/or gonadal steroid abnormalities can lead to the full range of symptoms seen in schizophrenia although it does remain feasible that in schizophrenia there is a particular pattern of abnormality developing at a particular time which is contributory to the development of schizophrenia.

The second reason why it is unlikely that reproductive hormones are causative, or even contributory, to the disease state of schizophrenia is the failure of replacement therapy to ameliorate schizophrenic symptomatology. There have been various studies of replacement therapy in schizophrenia which are outlined in Table 1.3. The design of these studies is somewhat haphazard but generally there was little evidence of a sustained clinical response to replacement therapy. The occasional dramatic response to sex hormone replacement reported in these studies no doubt reflects the tendency for a clinical response to any dramatic intervention in this condition. A caveat to this evidence against a hormonal aetiology of schizophrenia is that it is conceivable that replacement therapy was administered unphysiologically leading to a negative therapeutic response. For example it is known that for LHRH to be clinically effective, it needs to be administered in a pulsatile fashion (section 2.1.3). Continuous or high dose therapy can produce the opposite of the predicted effect.

TABLE 1.3

Investigators	Replacement therapy	Clinical details
Rosenzweig and Freeman (1942)	25 mg testosterone propionate and 300 units of "gonadotrophin"	20 chronic schizophrenics in double blind placebo study. Clinical improvement in 5 out of 10 patients (more psychomotor activity) 3 patients made worse. Greater sexual interest in patients on active medication
Hemphill and Reiss (1945)	Gonadotrophin releasing hormone 1000 U/day	22 chronic schizophrenics studied (open) 4 Recovered 8 No change 6 Improved 4 Worse
Bullmore and Coppen (1965)	Human chorionic gonadotrophin (HCG) 1500 U/day	3 adolescent schizophrenics (open) Clinical response not favourable
Brambilla et al (1974)	HCG 10,000 U/week	12 subacute schizophrenics (semi-blind) Apathy and affective response improved Synergistic with haloperidol

Summary of available studies on clinical effects reproductive hormone replacement in schizophrenics. There are some suggestions of a clinical response particularly in the symptoms of the defect state (see text for further comment).

1.3 Summary

1.3 In summary the aetiology of schizophrenia remains obscure. There are important indications that dopamine transmission may be enhanced perhaps in the context of structural CNS changes and there is some evidence of hypogonadism. This leads to the next section in which the relevance of studying pituitary hormone secretion in psychiatric disease as a method of evaluating central processes (particularly neurotransmitter function) is evaluated.

CHAPTER 2

Neuroendocrinology: an outline of current knowledge with special reference to its application to psychiatric illness

Neuroendocrinology is the area of research in which close attention is paid to the secretion of hormones from the central nervous system and their effects on the brain itself and on the rest of the body. There are two main areas of interest 1) The hypothalamo-pituitary axis and 2) The pineal gland.

Studies of pineal function in schizophrenia have been carried out by the author but are not further described in this thesis, because of constraints of space. A paper is attached (Ferrier et al, 1982) which reviews this area of research and its implications.

2.1 The hypothalamo-pituitary axis (HPA)

2.1.1 Historical aspects

Early in the twentieth century clinicians recognised that disease of the hypothalamus and the pituitary were related following the demonstration that gonadal deficiency could be produced by hypothalamic lesions which spared the pituitary. It was, however, not until the 1950s that Harris and co-workers (1952) in a series of elegant experiments clarified the relationship between the hypothalamus and the pituitary. They demonstrated that transection of the stalk between the hypothalamus and the pituitary caused a loss of sexual function in rats which was restored if blood vessels regenerated in this area, but not if this was prevented, and that the blood flow in

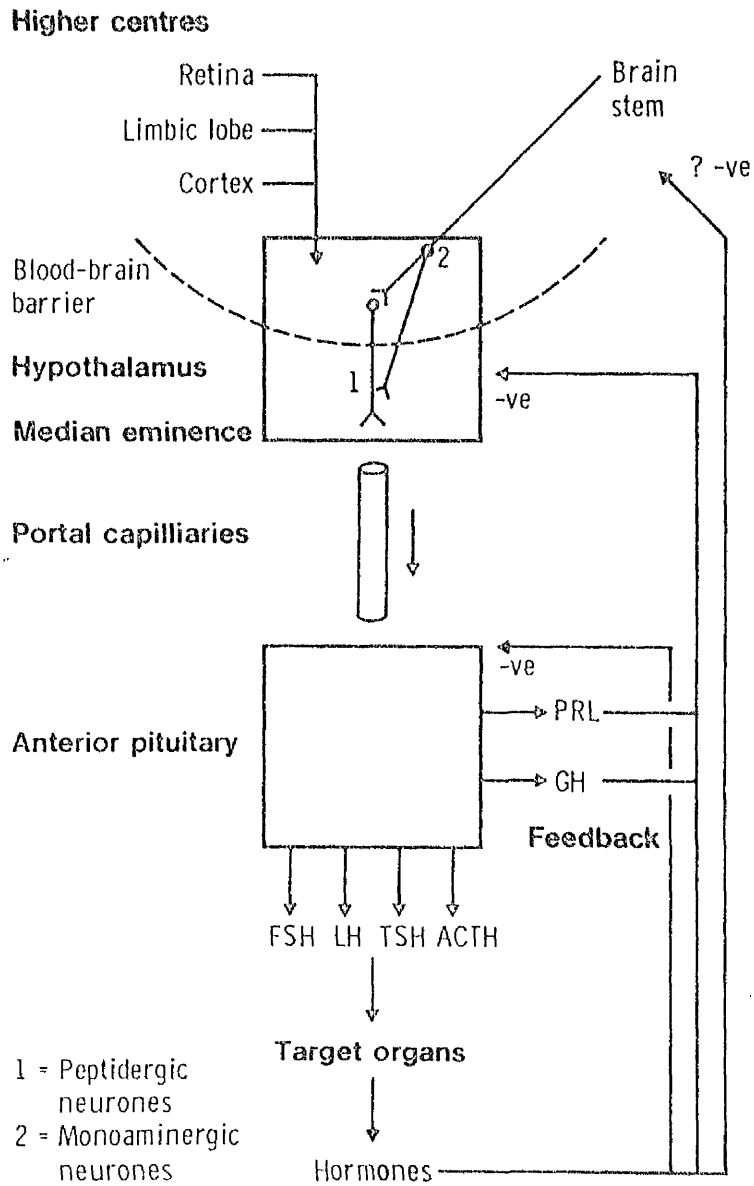
these vessels was from hypothalamus to the pituitary (Harris and Jacobsohn, 1952). Subsequently Halzasz and Knigge independently confirmed that there is a region of the basal hypothalamus that contains trophic substances capable of maintaining the secretory function of the pituitary and the term hypophysiotrophic area was applied to this region. In the late sixties and early seventies a number of these hormones - the hypophysiotrophic hormones or releasing hormones - were identified and characterised principally by Schally, Guillemin and co-workers (Schally et al, 1973; Guillemin et al, 1971) (Table 2.1).

2.1.2 Anatomy of hypothalamo-pituitary axis

A schematic representation of the anatomy of this system is shown in Fig 2.1.

Hypothalamus

The hypothalamus can be divided into the medial and lateral portions. The medial hypothalamus contains the bulk of neuronal cell bodies controlling pituitary function, as well as important receptors of visceral function; whereas the lateral hypothalamus is part of a multineuronal, multisynaptic system connecting the limbic forebrain with the mesencephalon. The hypothalamus contains many nuclei which can be divided into three groups - periventricular, medial and lateral but whose detailed description is beyond the scope of this work. Based on the pituitary micro-implantation studies it appears that the cell bodies of origin of the releasing hormones lie in a cresentic zone at the base of the 3rd ventricle and extend into the medial basal hypothalamus,



Schematic representation of the hypothalamo-pituitary axis showing interplay of central, peptidergic and monoaminergic influences at the hypothalamic level. Putative systems of feedback control are also demonstrated (see text for further details)

particularly the large and important arcuate nucleus. From this area neurones form the tuberohypophysial tract which sweeps round the 3rd ventricle and enters the median eminence. This is a highly vascular area that makes up the contact between the endings of the hypophysial-portal circulation. This area is characterised histologically by empydemal cells, capillary

structures and interstitial spaces. It is known that this area has a poorly developed blood-brain barrier.

Monoaminergic innervation of hypophysiotrophic neurones

The biogenic amines (in particular noradrenaline (NA), dopamine (DA) and serotonin (5-HT)) play a crucial role in modulating the secretion of the anterior pituitary trophic hormones, although they do not act directly on the pituitary itself (Fuxe and Hokfelt, 1969). The major pathways of hypothalamic innervation are outlined in Fig 2.1. NA neurones arise principally in the locus coeruleus and caudal medulla and ascend in the medial forebrain bundle to innervate many structures including several separate hypothalamic nuclei and the median emminence. 5-HT neurones innervate the hypothalamuc (particularly the supracharismatic nucleus) and originate in the raphé nuclei of the pons and mesencephalon. It has been shown by lesion experiments that 5HT and NA innervation of the hypothalamus is almost exclusively derived from these extrinsic pathways. On the other hand it would appear that the DA innervation of the hypothalamus is largely intrinsic. The neuronal cell bodies of the hypothalamic DA system - (the tuberoinfundibular DA (TIDA) neurones) - are principally located in the arcuate nucleus and their processes end directly in the median emminence. There may be some extrinsic DA innervation of the hypothalamus via the incerto-hypothalamic tract (Bjorklund, 1975).

Connections with other CNS structures

It is known that there are functional and anatomical connections between the hypothalamus and the limbic system (amygdala and hippocampus - via the medial forebrain bundle), the retina (via the direct retinocharismatic tract), the septum, the cortex, the midbrain, the reticular formation, the peri-aqueductal gray substance of the midbrain and the pineal. The details of the anatomy of these connections are not germane to the current studies but there are some important functional aspects which will be discussed below.

Pituitary hypophysial-portal capillaries

This mesh of capillaries transports hypophysiotrophic hormones from the hypothalamus to the pituitary and are thus crucial to the control of the HPA. CSF communication also exists between pituitary and hypothalamus and contributes, to an uncertain degree, to this control. It is believed that the flow of CSF is retrograde.

Pituitary

The pituitary gland is divided into three main parts, the anterior and the posterior pituitary and the intermediate lobe. The main hormone secreting portion of the anterior pituitary (adenohypophysis) is the pars distalis and this is the primary source of the six well known pituitary hormones (see Table 2.1). Older classifications of cell types in the adeno-hypophysis are now obsolete and it is known that many different cell types co-exist in the pituitary. There are also important receptors on the pituitary and their significance is alluded to below.

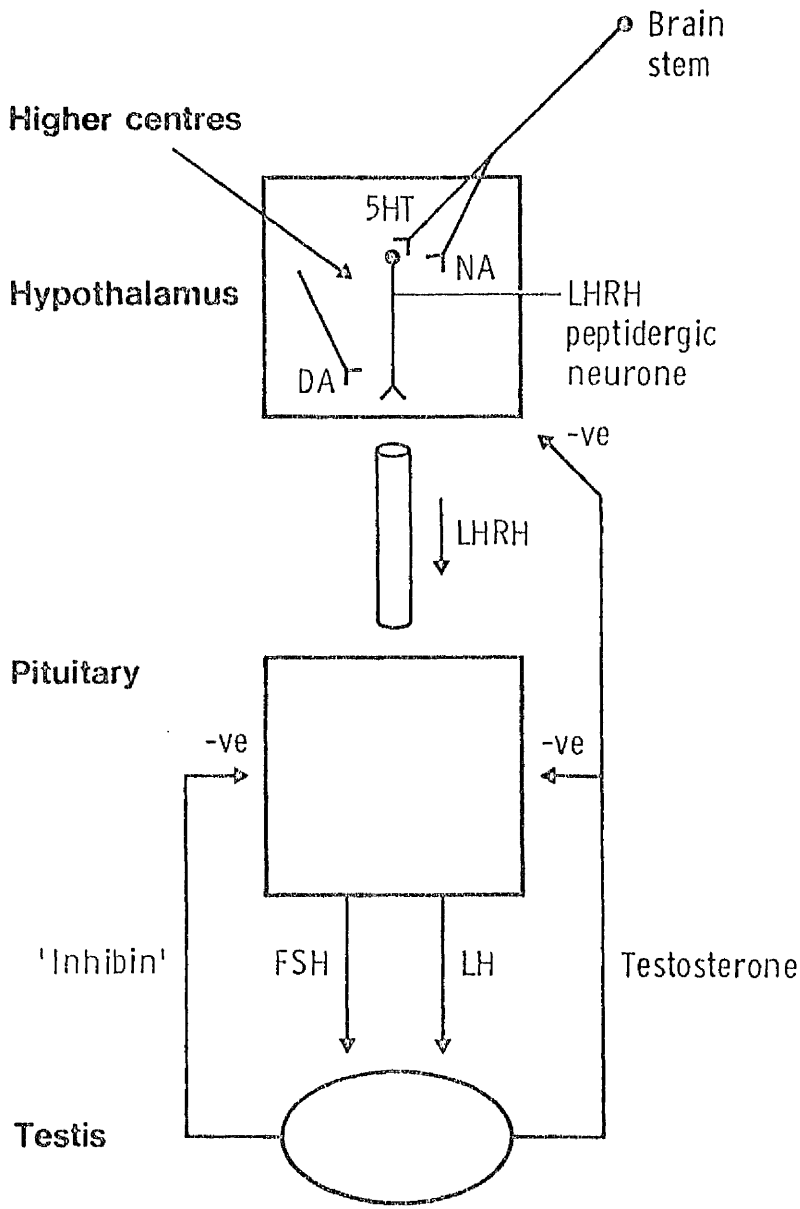
2.1.3 Physiology of the hypothalamo-pituitary axis

The control of pituitary hormone secretion is complex. An outline of the factors which control each pituitary hormone is given in Table 2.1 and is detailed for each individual hormone below.

1) Luteinizing hormone (LH). Secretion of LHRH by hypothalamic LHRH-peptidergic neurones constitutes the final common pathway of control of LH secretion and LHRH is involved in the many aspects of LH regulation. LH levels vary markedly with the age and maturity of the individual for example large changes in LH secretion are seen at puberty, during the menstrual cycle and at the menopause. Feedback from gonadal steroids (Fig 2.2) is a very important modulator of these changes in LH secretion but it is also recognized that influences from higher centres play an important role.

Under basal conditions in both men and women, pituitary secretion of LH is episodic (Boyar et al, 1972). The frequency of these episodes of secretion of LH varies from 12 to 24 per day and each is followed by a gradual decline to baseline compatible with the half-life of LH (Santen and Bardin, 1973). It would appear that these episodes of LH secretion are prompted by a bolus of secretion of LHRH from the hypothalamus and this is supported by the finding of rapidly changing LHRH levels in the peripheral blood synchronous with the changes in LH (Martin et al, 1977). Pituitary responsiveness to LHRH is maintained only to pulsatile LHRH administration and is lost to continuous LHRH exposure (Jacobsen et al, 1980). There is no diurnal rhythm of

FIG 2.2



Schematic diagram of gonadotrophin control system in men, showing interactions of neural and hormonal feedback controls. Secretion of testosterone by the testis is stimulated by LH: maturation and growth of the tubule by FSH. A newly discovered peptide secretion of the testis, "inhibin" is believed to be secreted by tubular epithelium and to exert a direct inhibitory effect on FSH secretion. It is not known whether inhibin affects the hypothalamus directly.

level. The most important hypothalamic nuclei in maintaining basal and pulsatile LH secretion is the supracharismatic nucleus which receives inputs from many sources notably the retina and brain stem. There is much less certainty about the function of the LHRH containing neurones in the remainder of the hypothalamus and in extra-hypothalamic areas which have been described in recent immunohistochemical studies in animals and man (Barry et al, 1977). The main LHRH containing fibres in the CNS are in the septum, anterior hypothalamus, midline perventricular structures and in the brain stem. It has been suggested that these peripheral LHRH pathways may mediate some of the behavioural effects of LHRH administration (such as mating behaviour) but this is not supported by direct evidence. Indeed the importance of afferent pathways (including monoamine containing neurones) entering the hypothalamus in the regulation of LH secretion is uncertain in humans. In the rat, lesions which isolate the hypothalamus disturb episodic LH secretion and the LH surge whereas in the rhesus monkey, both the tonic and episodic LH secretory mechanisms appear to be localized within the medial basal hypothalamus (Martin et al, 1977).

There is considerable evidence in experimental animals that the LHRH hypophysiotrophic neurones are regulated by biogenic amines although the precise details of this regulation are still unclear and a matter of controversy. A summary of the better recognized influences is given in Table 2.1. It has been demonstrated by immunohistochemical co-localization studies that there is a close relationship between LHRH containing neurones

TABLE 2.1

ıitary ıone	Hypothalamic Releasing Hormone	Physiological Stimuli	Role of putative neurotransmitters				
			Da	Na		5HT	Others
				α	β		
.actin	Prolactin inhibiting factor, P.IF ? Prolactin releasing factor/?TRH	Suckling +ve Stress +ve Sleep +ve Pregnancy+ve	- ve	——	——	+ ve	opiates + ve ? ACH
ıth tone	Growth hormone release inhibiting factor (somatostatin) growth hormone releasing factor	Stress +ve Sleep +ve Glucose↑ -ve	+ ve	+ ve	- ve	+ ve	opiates + ve GABA - ve
l	Corticotrophic releasing factor (CRF)	Sleep + ve Stress + ve Cortisol ↑- ve	- ve (?)	+ ve	- ve	- ve	opiates - ve
	Luteinising hormone releasing hormone (LHRH) ? LHRH	Puberty + ve Testosterone/oestrogen - ve Monopause+ ve	- ve (?)	+ ve(?)	——	- ve(?)	opiates -ve -ve ACH
	Thyrotropin releasing hormone (TRH) (somatostatin -ve	Cold +ve Cortisol↑-ve +ve Stress Oestrogen+ve T_4/T_3↑ -ve	- ve	——	——	- ve	opiates - ve

ACH = Acetylcholine
Da = Dopamine
Na = Noradrenaline
5HT= 5 hydroxytryptamine

Control of anterior pituitary hormone secretion.

and DA containing neurones in the median emminence (McNeil and Slalek, 1980). It would appear that DA can stimulate LHRH release from hypothalamic fragments in vitro. However subsequent studies have revealed that this is a complex phenomenon and that DA also plays a role in the degradation of LHRH in vitro (Marcanno de Cotte, 1980). The results of in vivo experiments of the role of DA on LH secretion yield similar conflicting results.

Pharmacological studies (McCann et al, 1977) demonstrated that DA directly injected into the ventricles causes release of LH. However the immunofluorescence studies of Fuxe et al (1969) revealed that DA levels in the hypothalamus and LH secretion vary inversely in such a way that these authors proposed an inhibitory role for DA in LH (and by extrapolation) LHRH secretion. The role of NA on LH secretion is probably facilitatory. Fuxe and co-workers (1969) noted increased turnover of NA at the time of ovulation and increased LH secretion. Blockade of NA synthesis leads to reduced LH secretion in response to a variety of stimuli (see Muller et al, 1977 for review).

A similar situation holds for the role of 5HT on LH secretion. Destruction of the raphe nuclei in the rat produces a striking reduction in pituitary LH secretion which has been shown to relate to changes in the 5HT content of the hypothalamus (Wuttke et al, 1979).

The role of biogenic amines in LH secretion in man is uncertain. Direct experiments such as outlined above of course

cannot be performed in man and accordingly evidence for the role of neurotransmitters in anterior pituitary hormone secretion (APHS) must rest on observations made in pharmacological studies which are outlined in the following section (2.1.4).

2) Follicle-stimulating hormone (FSH). There is much less known about the physiology of FSH secretion than that of LH secretion. It is not even certain if there is a separate FSH releasing factor. It is clear that under most circumstances there is a clear and close relationship between FSH and LH secretion implying a common releasing factor but there are also some striking discontinuities. The biochemical evidence for a separate releasing factor, though not extensive (Kastim et al, 1972), is outside the scope of this work.

The secretion of FSH shows a similar episodic pattern of secretion as LH although the size and frequency of epsidoes is much reduced and therefore more difficult to study. In general most workers find a delayed episode of FSH secretion following a LH secretory burst.

The role of biogenic amines in influencing FSH secretion appears to be minimal. In general, most of the effects of DA, NA and 5HT neurotransmitters which have been described above for LH also apply to FSH but the effects are weak and often not statistically significant. Feedback effects from gonadal steroids are of much greater importance and are outlined in Table 2.1

3) Prolactin (PRL). It is known that many stimuli influence PRL secretion (Table 2.1). Although many neural stimuli can induce PRL release, secretion is tonically inhibited by the

hypothalamus. Thus PRL secretion is increased by any process that interferes with hypothalamic-pituitary continuity. This means that the incubated pituitary releases large quantities of PRL in vitro. This has allowed considerable research into PRL secretion at the pituitary membrane level.

Prolactin-inhibiting factors (PIF) has been isolated from hypothalamic extracts and in pituitary-portal blood but the chemical nature of PIF is not known. DA has a direct inhibitory effect on PRL release in vitro, DA has been detected in pituitary-portal vessels (Ben-Jonathan et al, 1977) and in inhibitory hypothalamic fragments (in which inhibition is abolished by elimination of catecholamines) (McLeod et al, 1974). All these observations, combined with the presence of a high concentration of stereo-specific DA receptors in the pituitary but not in the hypothalamus (Brown et al, 1976), have lead to the hypothesis that DA itself is the physiological PIF. It has been found that hypothalamic extracts also contain a prolactin releasing factor (PRF) but its nature is unclear. Since thyrotrophin releasing hormone (TRH) releases PRL in low doses and binds to lactotrophes (PRL secreting cells) it has been put forward as a candidate for PRF. There is evidence however, for discontinuity in PRL and TSH secretion in certain clinical situations e.g. sleep and suckling. In addition there is evidence that TRH can be separated from PRF enzymatically and chromatographically.

PRL unlike other pituitary trophic hormones lacks a specific target gland with which to interact in a negative

feedback loop. Instead PRL secretion appears to be self regulated by a short loop mechanism. PRL administration dramatically increases DA turnover in the hypothalamus and this is evidence that this effect is mediated physiologically via retrograde CSF transport (Gudelsky et al, 1976).

A number of factors are known to influence PRL secretion. Oestrogenic hormones have a striking effect on PRL secretion which is thought to be a direct effect on the lactotrophe and not mediated by the known effect of oestrogens on DA turnover. A variety of stresses (Table 2.1) cause significant elevation of plasma PRL levels (Noel et al, 1972). Most studies have examined the effect of physical stressors and little is known of the PRL response to 'psychological stress'. A teleologically satisfactory rationale for stress induced PRL secretion is not apparent. Sleep induced PRL secretion has been demonstrated (Sassin et al, 1972). There are episodic bursts of PRL secretion during sleep which are not related to specific phases of sleep. PRL secretion is maximal at 4 - 6 a.m. and does not return to baseline till about 2 hours after waking. The role of PIF and/or DA in these sleep associated episodes of PRL secretion is uncertain, particularly in man. The pathways involved in mediating the influences of suckling, stress and sleep on PRL secretion are unknown. Lesion experiments in animals have shown that all forms of stimuli converge in the hypothalamus via an anterior route since most responses can be blocked by lesions in the preoptic region. There is some evidence that the stress response is mediated via the hippocampus

and amygdala.

Extensive evidence indicates that the monoamines, DA and 5HT are important in the control of PRL secretion in animals and man (McLeod, 1974). The evidence for the importance of DA is discussed above in the context that it may be the PIF substrate and the pharmacological evidence for the importance of dopaminergic drugs in humans is reviewed in the next section. Intraventricular administration of 5HT stimulates PRL secretion in rats (McCann et al, 1977). Presumably this effect is mediated via some CNS mechanism since 5HT does not directly affect PRL secretion in vitro. The situation is however complex because 5HT blockers (e.g. methysergide) stimulate PRL secretion in rats although they block the suckling induced rise in PRL. NA lowers PRL only after prolonged infusion, blockers of NA synthesis do not have any effect on PRL levels and NA and precursors are ineffective on pituitary cultures (Muller et al, 1977). On the other hand acetylcholine (ACH) does affect PRL secretion in vitro. However the role of ACH in PRL secretion is uncertain since cholinergic agonists and antagonists both appear to potentiate PRL secretion (Muller et al, 1977 for review).

4) Growth hormone(GH). The secretion of GH is precisely regulated by a complex interaction of stimulatory and inhibitory neural influences. This control is achieved by at least two hypothalamic hormones GH-releasing hormone (GRH) the structure of which is still unknown and GH-inhibiting hormone or somatostatin (SRIF) which has been isolated and structurally identified.

The actions of GH are numerous and include stimulation of growth, regulation of lipolysis and promotion of cellular uptake of amino acids. The pattern of GH secretion in humans is characterised by low (often undetectable) levels punctuated by pulses of GH secretion which are often of considerable magnitude, have a strikingly acute profile and are followed by a decline compatible with the known half-life of the hormone. It has been suggested that these surges are the result of primary neural activation of GH release.

It is known that GH secretion is related to glucose, protein and fat metabolism although changes in these metabolic pathways apparently do not account for the pulses of GH secretion described above. Glucose metabolism is the most important of these influences - rapidly decreasing glucose levels are a powerful provocative stimulus to GH secretion whereas glucose excess can block a number of agents which provoke GH secretion.

A number of physical agents e.g. pain, trauma, pyrogens are known to elevate GH secretion in man. Stress effects on GH secretion are however very complicated and seem to vary depending on the type of stress (Brown et al, 1978). It is known that most GH secretion occurs at night. Large episodes of secretion occur, particularly in children and are related, though not directly, to slow wave sleep (Lucke and Glick, 1971). It would appear from neuropharmacological studies that the regulation of sleep related GH secretion is different from that during the day and is dependant on 5HT metabolism (Brown et al,

1978).

The neural pathways involved in the regulation of GH secretion have been extensively studied in experimental animals and much detail elucidated. How much of this information applies to man is uncertain and there is evidence of important species differences in pharmacological responses (Brown et al, 1978). It would appear that a ventro-medial nucleus-median emminence pathway is important in GH secretion but that there is also an inhibitory SRIF containing pathway from the anterior hypothalamus (preoptic area). SRIF containing neurones have been found with an extensive distribution in cortex and limbic areas but their role in GH regulation is unknown. Simulation of a large number of CNS regions (e.g. ventral tegmental area, brain stem) have effects on GH secretion, the best recognized of which is the facilitatory role of the hippocampus and the dual role of the amygdala (Martin et al, 1977). The physiological significance of these observations is however uncertain.

Secretion of GH releasing and inhibiting hormones and the GH secretion induced by stimulation of extrahypothalamic structures is regulated by aminergic neurones acting at the hypothalamic level. Based on pharmacological responses it would appear that DA is facilitatory to GH secretion (see section 1.4). NA is thought to be a very important regulator of GH secretion and the evidence for a role in man is discussed in the next section.

5) Thyroid stimulating hormone (TSH). The hypothalamo-pituitary-thyroid axis represents an ideal example of a negative

feedback self regulatory system brought about by the interaction of at least three groups of hormones. Hypothalamic thyrotrophin releasing hormone (TRH) stimulates the release of TSH by the pituitary which in turn activates the thyroid to release thyroxine (T^4) and tri-iodothyronine (T_3).

TSH levels fluctuate to a small degree throughout 24 hours - with higher levels in the evening - the setpoint is principally controlled by the interplay of TRH and the level of T^4 and T_3. A number of physiological processes influence TSH levels though only within the limit of feedback control (Table 2.1). Stress in general inhibits TSH but this may reflect a feedback from increased thyroid hormone secretion (Mason, 1968). High levels of cortisol and GH appear to inhibit and oestrogen to enhance TSH secretion.

It is clear that the hypothalamus is essential for maintaining basal TSH secretion. A large number of hypothalamic nuclei are involved in TRH secretion and thereby TSH regulation. The role of extrahypothalamic structures in the regulation of TSH secretion is much less clear. It would appear that stimulation of the hippocampus and amygdala respectively enhance and inhibit TSH secretion. TRH and TRH receptors are distributed in extrahypothalamic sites and while there is increasing evidence of the behavioural effects of extrahypothalamic TRH, its role in TSH regulation is unknown (Krulich, 1982).

The physiological role of monoamines in the regulation of TSH secretion is much less studied and understood than for other pituitary hormones. DA and not NA releases TRH from

hypothalamic tissue in vitro but the suggestion of a facilitatory role for DA in TSH is contradicted by pharmacological studies in man (section 2.1.4). 5HT inhibits TSH secretion in vitro and the inhibitory effect of 5HT on TSH is confirmed in in vivo studies (Krulich, 1982).

6) Adrenocorticotrophic hormone (ACTH). Neural control of ACTH is responsible for its characteristic secretion pattern which leads to corresponding changes in secretion of the glucocorticoids from the adrenal cortex in phase with the daily activity cycle and the increased output that occurs during emergencies. Several steroids, some with sex-hormone activity, are secreted by the adrenal in response to ACTH but the principal one is cortisol (the remainder being outside the scope of this work).

Studies using indwelling catheters have revealed that ACTH is secreted episodically for brief periods of time and that these are large bursts of secretion in the later stages of sleep (around 6 a.m., unrelated to any particular phase of sleep). This leads to a corresponding pattern of cortisol secretion with low levels in the late evening and highest levels aroung 6 - 8 a.m. The rhythm of ACTH is under neural control. Negative feedback from cortisol plays little role in the modulation of the ACTH rhythm.

The ACTH-cortisol axis is activated by a wide variety of stressful stimuli. An attempt to define "stress" soon leads to crucial and difficult questions as to the nature of homeostasis. As was discussed in chapter 1.2.5 and was pointed out by Mason

(1968) psychological factors (e.g. arousal, novelty, uncertainity) may be the common stimulus for the adreno-cortical response, although there is much controversy and confusion in this area of research. Suppression of ACTH release can be brought about by the administration of exogenous steroids (e.g. dexamethasone) and it would appear that the neural origin of some stresses is powerful enough to overcome this suppression.

The regulation of ACTH secretion by the hypothalamus is somewhat complicated and is subserved by separate areas. On the basis of lesion experiments it would appear that the anterior part of the hypothalamus is responsible for the diurnal rhythm of ACTH secretion and that the stress response is mediated via the posterior basal hypothalamus. The final common pathway for ACTH secretion is corticotrophic releasing factor (CRF) secreted by the median emminence and whose structure is so far unidentified (summer, 1982). It is known that inputs from suprahypothalamic structures are important in ACTH regulation. Inputs affecting ACTH secretion from the brain stem, limbic areas and septal nuclei are recognized (Weiner and Ganong, 1978). ACTH is found in several brain areas and the injection of some analogues of ACTH has behavioural effects.

The analysis of the central neurotransmitter control of CRF-ACTH secretion has proven to be the most difficult of all the anterior pituitary hormone systems. It would appear that there is a facilitory cholinergic input at the level of the final CRF-containing neurone (Muller et al, 1978). The issue of the role of catecholamines in the control of ACTH remains

unresolved - particularly in man (see section 2.1.4): conflicting evidence points to an inhibitory, an excitatory or no role for each neurotransmitter. It is virtually certain that DA plays no role in ACTH regulation. The most commonly held view is that 5HT is facilitatory to most physiological ACTH secretory episodes (Checkley, 1980) and there is an inhibitory noradrenergic control of stress related ACTH secretion mediated by β adreno-receptors and opposed by α adrenoreceptors (Nakai et al, 1973).

2.1.4 Pharmacology of the hypothalamic-pituitary axis

An outline of the main changes in pituitary hormone secretion following the administration of neuropharmacological agents in man is given in Table 2.2. Important observations germane to this study are outlined below. It is important to recognize that despite considerable knowledge of the neuro-transmitter modulation of H-P secretion and of the pharmacology of drugs the site of action of a drug in an individual hormone response is by no means certain.

a) Dopamine (DA)

1) DA agonists. There are several drugs in this category and their principal modes of action are outlined in Table 2.2. Apomorphine (APO) is a direct agonist on DA receptors although there is considerable evidence (see chapter 7.1) that at low doses it preferentially stimulates pre-synaptic DA receptors ("autoreceptors") leading to an effective reduction in DA neuro-transmission. Whether this phenomenon is involved in the pituitary hormone response to APO is not clear: most evidence indicates a uniform dose-hormone response curve (Cleghorn et al, 1982).

TABLE 2.2

ACTIONS OF DOPAMINE AGONISTS USED IN ENDOCRINOLOGICAL RESEARCH

DRUG	
1) Apomorphine	Potent DA receptor agonists, effective on DA sensitive adenylate cyclase system, ? selectively labels presynaptic DA receptors, decreases in DA turnover, ? increases 5HT turnover.
2) Ergot derivatives	
a) bromocriptine b) lisuride c) lergotrile	High affinity for DA receptor sites, no stimulation of DA-sensitive adenylate cyclase system in vitro, reduce DA turnover. Probable effect on 5HT receptors but ? effect on 5HT turnover.
3) L-DOPA	Increases synthesis of DA and NE and 5HT.
4) Amphetamines	Indirect presynaptic agent - releases biogenic amines - NE and DA and blocks re-uptake - increase in turnover of DA, NE and ? 5HT.

Pharmacological profile of dopamine agonists used in neuroendocrine studies of psychiatric patients

APO administration provokes GH secretion and suppresses PRL secretion (Lal et al, 1973; Ettigi et al, 1975). Baseline effects are important in this response: PRL suppression is marked when PRL levels are high and reduced when PRL levels are low (Langer et al, 1979); GH response to APO is reduced in cases with high basal GH levels (Cleghorn et al, 1982). Since there is discontinuity between the emetic effects of APO (caused by stimulation of DA receptors in the medulla) and its hormonal response and since APO administration has no effect on ACTH secretion, it is generally held that the hormonal effects are pharmacologically specific rather than stress related. GH and PRL responses to APO are blocked stereospecifically by DA receptor blockers (Lal et al, 1977). APO appears to have no consistent effects on LH, FSH, TSH or ACTH secretion.

The ergot derivatives (bromocriptine, lisuride and lergotrile) have a slightly different pharmacological profile but somewhat similar endocrine effects as APO. These drugs are more specific agonists at DA2 receptor (Table 2.2) and this is the predominant type of DA receptor on the pituitary surface (see chapter 1 section 2.3 for outline of this classification) which argues in favour of a direct pituitary site of action for these drugs. However the evidence for this is not complete and indeed there is evidence in favour of a hypothalamic site of action for these drugs and for APO (Brown et al, 1982). There is a dispute, which may be a theoretically important one, regarding the effect of bromocriptine and DA infusions on LH secretion (Johnstone and Ferrier, 1980).

L-DOPA is a drug with weak and partial actions as a DA agonist. The effect of L-DOPA on PRL secretion is small and inconsistent (Lal et al, 1975) and the GH response is blocked by 5HT antagonists which make it a poor drug to use in testing DA receptor function. A similar argument applies to amphetamine - weak and inconsistent effects on PRL secretion are noted (Wells et al, 1978) and the GH response appears to be largely mediated via noradrenergic synapses (Checkley, 1980).

2) DA antagonists. The hormonal responses to this group of drugs has been intensively investigated both as part of basic science research and also because of their importance in psychiatric treatment and the need to monitor their administration. Administration of DA antagonists leads to a large and rapid secretion of PRL both in vitro (McLeod et al, 1974) and in vivo. It is generally held that this response is due to blockade of pituitary DA receptors and resultant loss of inhibitory DA tone. There is a close linear relationship between the DA blocking potency of neuroleptics in vitro and their PRL elevating properties (McLeod et al, 1975). It has been shown that the administration of neuroleptics leads to reproducible and dose-dependant elevation of PRL secretion in the individual subject (Langer et al, 1979) but that there is a large inter-individual variation. A ceiling of PRL secretion occurs after repeated or large doses of neuroleptics (Gruen et al, 1978). The question of tolerance to neuroleptics will be discussed in the subsequent relevant sections.

There is much conflicting data on the effect of DA

antagonists on growth hormone secretion. The basis of these differences appears firstly to be a significant species difference between primate and sub-primate (Weiner and Ganong, 1978) and secondly the varying α receptor blocking activity of different neuroleptics. It would appear that neuroleptics can reduce the GH response to many physiological stimuli but that they have no effect on basal GH secretion (Checkley, 1980 for review).

In general most studies (Beumont et al, 1974a, Cotes et al, 1978) have shown no effect of neuroleptics on LH secretion directly although a reduction in LH is believed by some authors to follow from neuroleptic-induced chronic hyperprolactinaemia (Glass et al, 1975). There are some instances of neuroleptics increasing LH where it was previously low. This phenomenon will be discussed more fully subsequently.

There is no conclusive evidence for an effect of neuroleptic medication on ACTH secretion in man. Recently small but clear-cut elevations in TSH secretion have been observed following the acute administration of the dopamine antagonist metoclopramide (MCP) (Healy and Burger, 1977).

b) Noradrenaline (NA)

1) Agonists. It appears that drugs of this type e.g. clonidine stimulate the release of GH but have little effect on PRL secretion. Amphetamine has been used extensively in research into psychiatric disorders. It would appear to act through α-adrenergic receptors to elevate GH and ACTH although clonidine which is a more specific α-adrenergic agonist does not elevate

ACTH secretion (Checkley, 1980 for review).

2) Antagonists. a) β receptor blockers have been used to treat schizophrenic patients. They appear to potentiate the GH and ACTH response to most physiological stimuli but to have no endocrinological effects alone (Checkely, 1980).

b) α blockers. α blockade inhibits the GH and ACTH response to a wide variety of stimuli but has little effect on PRL or LH secretion in man.

c) Acetylcholine (ACH). It has been suggested that anti-cholinergic medication causes elevation of PRL levels (de Rivera et al, 1976). This claim is important in research into the hormonal response of schizophrenic patients to neuroleptic drugs as drugs with this pharmacological property are frequently prescribed for schizophrenic patients as an adjunct to neuro-leptic therapy. However the evidence is not strong and the results of basic research on this point are conflicting (see chapter 6).

d) Opiates. There is currently controversy regarding the effect of opiate agonists and antagonists on GH and PRL secretion in man which is probably due to pharmcodynamic reasons. For the purposes of these studies it is only important to note that there is increasingly good evidence that opiate agonist decrease and opiate antagonists increase (Moult et al, 1981) LH levels and LH pulsatility and this may be particularly relevant where LH levels are low (Quigley et al, 1980).

2.2 Neuroendocrine studies of psychiatric illness

2.2.1 INTRODUCTION

This is a rapidly advancing field of study. These studies have two basic aims:- 1) the discovery of a hormonal marker for disease that will be useful as an index for diagnosis, treatment and prognosis and 2) the elucidation of an hormonal abnormality will aid understanding of any underlying neurotransmitter abnormality and hence aetiology. To use the American argot of the day - these studies are using pituitary hormones as a "window on the brain". How well the physiology and pharmacology of anterior pituitary hormone secretion stands up to this sort of analysis is outlined above and discussed in the appropriate sections.

The studies reported in subsequent chapters (4, 5, 6, 7) concern patients with a diagnosis of schizophrenia. An outline of neuroendocrine studies in other psychiatric conditions is first given. The diagnostic specificty of the hormone abnormalities reported is a major concern of this work and future studies. An outline of the more established hormonal abnormalities in various psychiatric disorders is given in Table 2.3.

2.2.2 Neuroendocrine studies in affective illness

a) Depression

This group of illnesses have been studied extensively from a neuroendocrine angle. The basic abnormalities detected are:- 1) Hypersecretion of cortisol. Extensive work has been done on this phenomenon which was first recognized in the late sixties. It has been observed that many depressed patients have elevated cortisol levels with a loss of the normal diurnal rhythm (Carroll et al, 1980). In addition the high levels of cortisol

TABLE 2.3

CONDITION	Dopamine Agonists: Bromocryptine (Bromo), L-DOPA (L-D) APOMORPHINE (APO)	α Noradrenergic Agonists: CLONIDINE	AMPHETAMINES
Normal (see text)	PRL ↓ GH ↑ ? LH ACTH →	GH ↑ PRL → ACTH → LH →	GH ↑ PRL? ACTH ↑ LH →
Schizophrenia	? enhanced GH ↑ and reduced PRL ↓ to APO in some acute patients blunted GH ↑ and PRL ↓ to APO in many chronic patients	–	Normal GH responses
Huntington's Chorea	enhanced GH ↑ to BROMO but reduced PRL ↓	-	--
Parkinson's disease	Reduced GH to BROMO Normal/ Reduced GH to L-D	–	--
Acromegaly	GH ↓ PRL ↓	-	–
Affective Illness	Normal PRL ↓ Normal GH ↑	? Reduced GH in depression	Reduced cortisol ↑ in depression Reduced GH ↑ in depression

Summary of neuroendocrine studies in neuropsychiatry with particular reference to studies with provocative agents.

in many cases fail to suppress normally on the administration of exogenous steroids e.g. dexamethasone. The dexamethasone suppression test (DST) has now been carried out on many hundreds of patients and is held by some workers to be highly selective for a group of patients with endogenous depression. It is also claimed that the DST is unaffected by phenomena such as anxiety and agitation or drug therapy and that it is able to predict treatment response and relapse (Carroll et al, 1980 for review). Claims of selectivity and specificity however are somewhat weakened by the finding of abnormal DST results in patients with anorexia nervosa (Gerner et al, 1981). Another problem is that basal ACTH secretion does not appear to be abnormal in depression.

2) Blunted TSH response to TRH. It is now well established that a proportion of depressed subjects have a markedly blunted TSH response to TRH administration in the presence of normal T3 and T4 and no clinical evidence of thyroid disease (Asnis et al, 1981). The incidence of this abnormality is about 33% of well diagnosed depressed patients. There is much argument about whether this is a trait or state dependant phenomenon. There is general agreement that the blunted TSH response does not exhibit a clear relationship to abnormalities of the DST.

3) Aberrant hormonal secretion to LHRH and TRH. It has been reported that in a group of depressed patients there was evidence of disturbed pituitary secretion in response to synthetic releasing hormones. Not only is the TSH response to TRH blunted in a number of cases (see above) but a number of unusual responses

e.g. LH and GH responses to TRH and PRL and GH response to LHRH have been reported (Brambilla et al, 1978; Maeda et al, 1975). These authors proposed a functional disconnection between the hypothalamus and the pituitary in depression.

4) Diminished pituitary secretion in response to insulin hypoglycaemia. A number of authors have demonstrated reduced GH and ACTH responses to insulin hypoglycaemia in depression (Checkley, 1980). Since there is a close concordance between the presence and severity of this abnormality and abnormalities of the DST, it is thought that these blunted hormonal responses are non-specific and that raised cortisol prevents an adequately provocative hypoglycaemia.

5) Reduced hormonal response to α-adrenergic agonists. There is some good evidence for a reduced GH response to clonidine in depressed patients which reverts to normal after amelioration of clinical state with electro-convulsive therapy (ECT). A similar blunting of the GH and ACTH response to amphetamine administration has been reported (Checkley et al, 1979). Amphetamine is thought to act mainly as an α-adrenergic agonist (see section 2.1.4).

6) Abnormal gonadotrophin secretion. There have been isolated and poorly substantiated claims of reduced LH and FSH secretion in depression (Brambilla et al, 1979).

One study on 3 post-menopausal depressed patients found a reduction in LH pulsatility (Altman et al, 1975) but the number of subjects studied precluded adequate statistical

evaluation of this phenomenon.

Summary of neuroendocrine studies in depression

As can be seen from the foregoing account several abnormalities of pituitary hormone secretion have been noted in depression. The outstanding problems are to what extent these abnormalities are specific for the diagnosis of depression and to what extent secondary factors (e.g. weight loss) may account for them. The pathophysiological basis of these abnormalities is still obscure: most evidence points to a hypothalamic abnormality and the possibility of an adrenergic and/or cholinergic deficit. Some of these issues will be addressed in subsequent sections.

b) Mania

Neuroendocrine studies on patients suffering from mania or hypomania are few although there are more studies available on patients with bipolar affective illness. These studies have recently been comprehensively reviewed by Whalley (1982). Cortisol, PRL and TSH secretion are poor discriminators between different types of affective illness. It does however appear that the GH response to DA agonists or α agonists is different between depressed patients and those patients with mania or a history of it. These responses are blunted in depressed patients but frequently enhanced in the latter group of patients. The pathophysiological significance of this finding remains to be determined.

2.2.3 Neuroendocrine studies in patients with anorexia nervosa (AN)

Amenorrhoea in female A.N. patients is invariable and there are clear indications of hormonal perturbation in these patients. LH and FSH secretion is low and loss of pulsatility of LH secretion has been demonstrated. Urine and serum levels of oestrogens are also markedly reduced (Marshall et al, 1979). The endocrinological picture is frequently compared with pre-pubertal girls and the endocrinological pattern goes through the stages of puberty on clinical imporvement and weight gain.

It is now more or less accepted that these hormonal abnormalities are mediated at the hypothalamic and not the pituitary level. The LH response to a bolus or continued infusion of LHRH is frequently blunted. However if LHRH is administered in frequent low doses, responsivity to LHRH and the normal episodic release pattern is soon restored (Valk et al, 1980; Jacobsen et al, 1980). The endocrinological status of those few male patients with A.N. has not, unfortunately, been studied.

These patients frequently have other hormonal abnormalities e.g. borderline T_4 levels with reduced T_3 levels and elevated GH levels. These abnormalities are closely related to weight loss and revert to normal on weight gain. Abnormalities of the DST in A.N. were alluded to in section 2.2.2. Hypersecretion of cortisol has also been demonstrated in A.N. and in normal subjects on a fast (Doerr et al, 1981).

2.2.4 Neuroendocrine studies on patients with organic psychiatric syndromes

Neuroendocrine studies on patients with organic mental

disorders (e.g. dementia, confusional syndromes) are few. The best conducted and controlled study demonstrated normal LH, FSH and PRL secretion rhythms in patients with senile dementia of the Alzheimer type (Touitou et al, 1981).

2.2.5 Neuroendocrine studies in patients with Parkinson's disease and other DA-related diseases

Several groups of workers have shown that the GH response to DA agonists is either reduced (Parkes et al, 1976) or reversed (Hÿppa, 1978) in patients with Parkinson's disease. The pathophysiological basis of this disease is degeneration of DA containing neurones. The typical pathological changes of the disease are found in the hypothalamus and reductions in hypothalamic DA content have been demonstrated (see references above) in Parkinson's disease brains. The GH response to DA agonists is most probably mediated at the hypothalamic level and so the abnormal responses discussed above are representative of the underlying pathophysiology of the disease.

The satuation is less clear-cut with Huntington's chorea - a disease in which cell bodies of the striatum degenerate leading to perturbation of GABA/DA interplay. In this disorder enhanced GH responses to DA agonists are predicted. There is one such report in the literature (Caranceni et al, 1977) but there are several conflicting and negative studies.

2.2.6 Neuroendocrine studies in schizophrenia

A summary of tne available neuroendocrine studies in schizophrenia is given below, taking each hormone in turn. Some of

this information has been discussed in chapter 1 from the aetiological point of view. These two different approaches are discussed in chapter 1.2.5 and again in chapter 8.

a) Prolactin (PRL). In view of the inhibitory influence of the tubero-infundibular DA (TIDA) system on PRL secretion, the DA overactivity hypotheses of schizophrenia predicts that PRL secretion will be low in patients untreated with neuroleptic drugs. Several groups of workers have investigated this question and there is general agreement that basal PRL levels are normal both in acute (Meltzer et al, 1974; Pandey et al, 1977) and chronic schizophrenia (Brambilla et al, 1976; Johnstone et al, 1977). This result can be interpreted in several ways, for example:-

1) The DA hypotheses of schizophrenia has no validity
2) DA overactivity does not extend or apply to the TIDA neurones
3) Feedback effects restore PRL levels to normal. These possibilities, together with supporting and confounding evidence, are discussed in the appropriate sections.

Other aspects of PRL secretion in schizophrenia have also been extensively studied. The most important of these is the PRL response to neuroleptic drug administration. It has been shown that there is no difference in the timing or the magnitude of the PRL response to acute neuroleptic administration between schizophrenics and controls (Gruen et al, 1978). Langer et al (1979) have reported that if DA is infused IV following the acute administration of neuroleptics to elevate PRL to approximately half maximal, the PRL suppression observed is greater in

schizophrenics than in controls. These authors suggested this is evidence of supersensitive DA receptors on schizophrenic pituitary glands. However it is obvious that there are considerable interpretative difficulties associated with this experiment which, in any event, requires replication.

The effects of chronic therapeutic neuroleptic administration on PRL secretion have been studied by several groups of reserachers in the hope that PRL secretion would prove to be a useful index or marker of central DA blockade. It has been found that in general there is a relationship between the dose of neuroleptic administered and associated increments of PRL secretion (Rao et al, 1980). However there are several important points to be noted regarding this relationship:- 1) The PRL response to neuroleptics varies markedly between male and female controls and between male and female schizophrenics (Cotes et al, 1978) so that it is important to examine these groups separately. 2) A ceiling effect develops such that subsequent administration of neuroleptics will not further elevate PRL secretion (Gruen et al, 1978). 3) There is a remarkable inter-individual variation in the PRL response to neuroleptics (Langer et al, 1977). 4) The chronicity of this relationship is uncertain as there is considerable debate as to whether or not tolerance to the PRL elevating effects of neuroleptics occurs. Meltzer et al (1976) reported that tolerance did not occur, but this study was over a relatively short time scale. Several studies have shown that over a prolonged period of neuroleptic administration (> 5 years) PRL levels return

towards the normal range particularly in elderly men (de Rivera et al, 1976; Huws and Groom, 1977).

There is also uncertainity about the relationship between neuroleptic administration, PRL secretion and clinical efficacy and side effects. With two possible exceptions all drugs which are known to be effective neuroleptics cause elevation of prolactin secretion. Clozapine elevates PRL to only a slight extent and is held to have anti-psychotic properties. Similarly the β blocker, propranolol, appears to be effective in high dosage in the treatment of schizophrenia but has little or no effects on PRL secretion (Johnstone and Ferrier, 1980 for review). Indeed while there is an overall statistical relationship between the acute PRL elevating potency of neuroleptics and their clinical efficacy (Langer, 1977) and some clinical demonstrations of a positive relationship between the antipsychotic effect of neuroleptics and the associated elevation of PRL secretion (Meltzer et al, 1976; Langer et al, 1977), it is clear that this relationship is not a strong one and does not necessarily apply to individual cases (e.g. a good clinical response to neuroleptics may be observed associated with a low increment in PRL secretion and vice versa).

Other clinical relationships between PRL secretion and neuroleptic administration that have been demonstrated are:- 1) a more marked elevation following neuroleptics of PRL in cases of tardive dyskinesia (Glazer et al, 1981) 2) a more rapid rate of fall of PRL on neuroleptic discontinuation in those cases with extrapyramidal side effects (Brown et al, 1979)

and 3) higher PRL levels in those patients with extrapyramidal side effects of neuroleptics (Kolakowska et al, 1975). All these studies require confirmation. Aspects of the above relationships are investigated in the present study.

Another dynamic test of PRL secretion that has been studied in schizophrenic patients is the PRL response to TRH. This was examined by Prange et al (1979) who found a normal response. This study will be discussed in critical detail in the subsequent relevant section.

Despite the normality of PRL levels in schizophrenia, mentioned at the beginning of this section, and this finding's seeming lack of support for the DA hypothesis of schizophrenia, investigators have continued to examine PRL secretion in schizophrenia looking for a clinical correlate of PRL secretion. One such finding was reported by Johnstone et al (1977) who clinically rated 16 neuroleptic unmedicated patients and measured PRL on two well-separated occasions and found a significant relationship between increasing positive (delusions and hallucinations) symptoms and decreasing PRL secretion. This finding emphasizes the importance of looking at the dynamic aspects of pituitary secretion. A similar finding has recently been reported by Kleinman et al (1982) who found a negative relationship between positive symptoms and PRL secretion only in those patients with normal ventricular size on CAT scan. This relationship was studied in some detail in the present studies.

b) LH and FSH secretion. As was discussed in section 1.2.5 (in the context of aetiological hypotheses) three separate

groups of works have noted reduced LH and FSH secretion in unmedicated male chronic schizophrenics (Shader et al, 1968; Brambilla et al, 1976; Johnstone et al, 1977). Further evidence that a degree of hypogonadism is manifest in a subgroup of schizophrenic patients was also outlined in the preceeding chapter.

The significance of these findings is not clear at the present time. Summarising the work of all three studies it is apparent that low LH and FSH are found only in a subgroup of patients and there is a degree of overlap with the normal range. It is clear that any defect of LH and FSH regulation in schizophrenia is only one of degree (the FSH and LH levels are not as low as those patients with absence of LHRH secretion (idiopathic gonadotrophin deficiency : IGD patients)). There is however evidence that reduced LH and FSH secretion may have some relationship to clinical state - both Shader et al (1968) and Johnstone et al (1977) found that those patients with the more disturbed psychopathology had the lowest FSH levels and Brambilla et al (1975) found that the therapeutic administration of haloperidol was associated with a return of LH and FSH to the normal range.

Single sample studies are an unreliable method of investigating hormonal secretion as the levels of most hormones, including LH and FSH, fluctuate widely (as was outlined in section 2.1.6) and steps were taken in the present study to overcome this problem.

c) GH and PRL response to dopamine agonists in schizophrenia.

It is accepted that basal PRL secretion is normal in schizophrenia (see section 2.3.7)) and there appears to be a similar acceptance that basal GH secretion is normal in schizophrenia (Cleghorn et al, 1982). The discovery that DA agonists administration produce reproducible and large changes in PRL and GH secretion (see chapter 2.1.4 for details) has provoked a number of research groups to investigate these hormonal responses in schizophrenic patients looking for evidence of a change in dopamine receptor sensitivity. As discussed in chapter 2.1.4 the most direct DA agonist and drug with the purest neurochemical and hormonal profile is apomorphine (APO). Studies with schizophrenic patients have also been performed with less direct DA agonists e.g. amphetamine and L-DOPA. The results of these studies are outlined in Table 2.4 and a synthesis of the studies with APO given below.

There have been 5 such studies involving a total of 94 patients of various clinical types with differing dose regimens. Generally speaking most groups of workers find that the GH response to APO is blunted in patients with "chronic schizophrenia" although there are many such patients with results in the normal range. The relationship between the clinical states of these patients (in terms of such phenomena as negative or positive symptoms and cognitive impairment) and the GH response has not been comprehensively studied. Similarly there is controversy as to whether DA receptor subsensitivity in chronic schizophrenics relates to either previous neuroleptic drug therapy or to subsequent neuroleptic drug response.

TABLE 2.4

Investigators	Patients Studied	Drug+ Dose	Response	Comment
Langer et al (1976)	21 Normals 8 Schizophrenics 17 Depressives	Amphetamine sulphate 0.1mg/kg	Normal GH response	Blunted GH response in depressed patients
Ettigi et al (1976)	17 Chronic schizophrenics 21 Controls	APO 0.75mg sc.	Blunted GH response Normal PRL response	Blunting related to drug therapy
Pandey et al (1977)	9 Acute schizophrenics 15 Chronic schizophrenics 8 Controls	APO 0.75mg sc.	GH response elevated in acutes and blunted in chronics	No relation to drug therapy
Rotrosen et al (1978)	10 Controls 17 Schizophrenics	APO 0.5mg sc. L-DOPA 500 mg po.	Blunted PRL and GH response to APO	Important baseline effects
Tamminga et al (1977)	10 Controls 10 Schizophrenics	APO 0.75mg L-DOPA 500mg po.	Blunted GH but normal PRL response to APO and L-DOPA	No relation to drug therapy or side effects
Rotrosen et al (1979)	21 Controls 26 Schizophrenics	APO 0.5mg sc.	Normal mean GH and PRL responses	Bimodal GH response

Summary of neuroendocrine studies with dopamine agonists in schizophrenic patients.

GH studies after APO in patients with schizophrenia of more recent onset or accompanied by acute symptoms have given potentially interesting results. It is now accepted that while acute schizophrenics as a group have normal GH responses to APO, they also (as a group) exhibit markedly more variable GH responses than the normal population (some patients exhibit grossly elevated GH levels after APO whilst others have little or no response). The clinical correlates of this divergence are unknown at present - at one time it was claimed that this response was a predictor of neuroleptic drug response (Pandey et al, 1977) but this has subsequently been retracted (Rotrosen et al, 1979). Recently Cleghorn et al (1982) have reported, in a small number of patients, that the GH response increases markedly in those patients who are in the process of relapsing. This raises the possibility that the GH response to APO is a state, as opposed to trait, marker of the schizophrenic process.

All studies of this type share the problem that the patients studied must be drug free: several of the above studies were performed after neuroleptics had been discontinued - sometimes for as short a period as 7 days. Animal studies have shown that neuroleptic withdrawal increases DA receptor sensitivity in striatal areas (Burt et al, 1977). The only comparable study on pituitary DA receptors indicated a reduced DA receptor sensitivity in vitro after drug withdrawl (Friend et al, 1977). However from the functional point of view there is evidence for increased DA receptor sensitivity after neuroleptic therapy in animals in that the PRL suppression after APO was

exaggerated (Lal et al, 1977). The PRL response to APO in schizophrenic patients appears to be comparable with that found in the normal population. However there are technical and practical difficulties in employing the PRL response to APO as a measure of DA receptor sensitivity. These are fully developed by Rotrosen et al (1979) and Johnstone and Ferrier (1980) and include:- 1) the narrow range between normal PRL levels and the lower limit of detection of the assay system 2) the floor effect beyond which even massive doses of the drug do not further suppress PRL secretion 3) the response does not exhibit adequate dose-response properties 4) the nausea and emesis that APO may produce is "stressful" which may produce endocrine effects.

Attempts to circumvent these theoretical and practical problems have been made in the present study (chapter 7).

d) Studies on adrenal and thyroid function in schizophrenia As was outlined in chapter 1 (section 2.5) intensive investigation of adrenal and thyroid function in schizophrenia was undertaken during the 1930s and 1940s (the era before neuroleptic drug treatment) and no convincing abnormality was identified (Kline et al, 1968). In more recent times several groups of workers have found normal levels of cortisol secretion in schizophrenia associated with a normal diurnal rhythm. Similarly normal T_3 and T_4 (Prange et al, 1979) and TSH levels (Johnstone et al, 1977) have been reported in schizophrenia. The TSH response to TRH appears to be normal in schizophrenia in distinction to depressive illness (Asnis et al, 1981).

2.2.7 Summary

Several pituitary hormone abnormalities have been detected in various psychiatric diagnostic categories. The most studied disease is depression where hypersecretion of cortisol, blunted TSH response to TRH and reduced GH response to α agonists have been demonstrated with varying rates of frequency. There is evidence that the DST test is a useful clinical marker although this rests heavily on the work of one group (Carroll and collaborators, An Arbor, USA). Whether these findings are epiphenomena or related directly to the pathophysiology of the disease remains to be clarified. There are striking abnormalities of pituitary hormones in anorexia nervosa which appear to relate directly to weight loss. Dementia does not appear to be specifically associated with any neuroendocrine abnormality. Abnormalities of neuroendocrine function have been noted in Parkinson's disease and Huntington's chorea but the extent they reflect the known changes in neurotransmitter functions in these diseases is disputed.

As regards schizophrenia, there is evidence for selective reductions in gonadotrophin secretion with some evidence that these impairments relate to clinical state. While it is probable that these changes are not causative (see chapter 1.2.5) their relationship to the underlying pathophysiological process of the disease remains to be clarified. PRL secretion appears normal in schizophrenia but there appear to be some relationships between clinical state and neuroleptic drug administration which could prove fruitful areas of research.

The GH response to DA agonists appears abnormal in several different ways in patients with schizophrenia and the relationship of this response to drug therapy and clinical state require further evaluation. These questions have been the focus of the present research which will now be described in detail.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Patient selection and classification

The studies reported here are principally on three groups of subjects:- a) chronic schizophrenic patients b) acute schizophrenic patients and c) controls. The exact details of the groups studied will be made clear in the description of individual studies (chapter 4 - 7) but an outline of the methods of patient selection are given below. Patients who do not fall into the above three categories (for example the few patients with affective illness and the small group of acute schizophrenics in remission) are described in detail in the appropriate sections.

3.1.1 Chronic schizophrenic group

This group were all long-stay patients of a neighbouring large mental hospital (Shenley Hospital, Herts). With the co-operation of the local consultants a survey of the population (hospitalised for more than a year) of this hospital was carried out (which has been reported in detail elsewhere (Owens and Johnstone, 1980)). A diagnosis of schizophrenia was made in those patients who conformed to the criteria of Feighner et al (1972). These criteria are fully described in Appendix (A). Operational criteria are applied to the casenotes (and so refer to symptoms at any point in the patient's history and not to the current clinical state). The diagnosis requires the presence of clearcut, unequivocal delusions and hallucinations in the presence of clear consciousness and in the absence of several other features for example alcoholism. In addition, to satisfy

these criteria, there must have been no return to the pre-morbid level of functioning six months after admission. These criteria therefore, to a certain extent, select out a group of patients with an unfavourable clinical response to hospitalisation and therapy. In any event this was ensured by the criteria of one year's continuous hospitalisation adopted by the survey. In practice all of the patients had been hospitalised continuously for a considerable number of years at the time of the present studies despite intensive attempts at rehabilitation and discharge. It is important to note therefore that the patients reported on here are at the poor end of the prognostic spectrum. The mental states of these patients at the time of study was rated separately (see section 3.2) and varied considerably.

In addition the patients were classified according to the Catego system of classification (Wing et al, 1974). The features of the illness at its worst were recorded by the application to the casenotes of the syndrome check list of the Present State Examination. A basic description of this techniques and an outline of the types of diagnostic groups that are thereby identified is given in Appendix (B). Items were recorded only where there was a clear description of the patients' mental state e.g. statements that the patient was hallucinated or incoherent alone did not qualify for a positive recording. Further details regarding the selection of this group of patients (e.g. ages, physical health and drug status) are described in section 3.1.4.

3.1.2 Acute schizophrenia

This group of patients were all inpatients in the research

ward of the Division of Psychiatry, Clinical Research Centre. A clinical diagnosis, on the basis of the presence of delusions and hallucinations, in clear consciousness and not based on affect, was first made. Only patients who had developed new symptoms during the past month were included. A full interview of the Present State Examination was carried out (Wing et al, 1974) which involves rating 90 symptoms and 50 signs. These interviews were carried out by research psychiatrists specifically trained and experienced in the use of this technique. Only patients in whom at least one feature of nuclear schizophrenia (essentially the presence of a Schneiderian first rank symptom) was unequivocally present were included. Some of these patients were first-episode cases; others had had one or more relapses - details are outlined in individual studies.

Only a few patients deemed suitable for study on the above criteria proved untestable because of their clinical condition. Problems included refusal to participate, excessive suspiciousness, hostility and physical aggressiveness.

3.1.3 Control subjects

A total of 68 controls were studied. They were selected on the basis principally of age-sex matching with the various schizophrenic groups. Groups selected for a particular study are described in the individual studies. An outline of the basis of selection is given below. Controls were of various types as follows:-

1) Young control group - 16 healthy male laboratory or hospital staff.

2) Elderly control group was made up of four subgroups (a mixture of patients from these subgroups made up a control group for a particular study).

a) 15 healthy normal controls consisting of hospital staff and volunteers.

b) 8 patients attending medical clinics at Northwick Park Hospital with non-endocrine, non-metabolic disease e.g. ischaemic heart disease, peptic ulceration, bronchitis. These were included because it is considered unwise to select control groups on the basis of health as this can induce bias.

c) 10 patients attending the psychiatric clinic with a diagnosis of mild reactive depression, anxiety or personality disorder. Psychotic illness was excluded by clinical examination, prolonged outpatient observation and application of the Feighner criteria to casenotes. These patients to some extent provide a control group for the non-specific effects of psychiatric disease.

d) 16 patients who were inpatients in Northwick Park Hospital admitted for minor elective orthopaedic surgery (e.g. removal of pins, ganglion, contractures).

e) 3 long stay patients of Shenley Hospital with a diagnosis of affective illness by the St. Louis criteria (Feighner et al, 1972) but whose mood was normal at the time of study.

3.1.4 Additional points on patient selection

(1) Sex/Age

With the exception of a few patients, described in chapter

6, all the patients studied were male. This was to avoid the complications of the variable effects of the menstrual cycle on pituitary hormone secretion and the additional difficulties of the effects of either amenorrhoea or the menopause on hormonal levels. There is good evidence that post-menopausal females respond to provocative hormonal tests in a qualitatively different way from menstruating females. Patients between the ages of 16 and 69 were considered for selection.

(2) Consent

All the patients and controls studied gave consent for the procedures undertaken. In the case of all of the controls and the majority of the schizophrenics this consent was informed i.e. the subjects were aware of the issues involved, their right to refuse and the fact that the procedures were not part of routine investigation or treatment. A few of the schizophrenics were unable to grasp this proposition but co-operated willingly - in these cases consent was obtained from the nearest relative. In a very few cases the full experimental protocol could not be completed due to non-compliance. These patients are alluded to in chapter 6.

(3) Physical health

A clinical history was taken from all patients and controls and a basic physical examination performed. All patients with major endocrine, metabolic or neurological disease were excluded. Schizophrenics with a history of epilepsy or leucotomy were excluded. A number of the chronic schizophrenic patients had evidence of some non-psychiatric disease e.g. peptic ulceration,

osteoarthritis, mild hypertension, vascular disease. It was therefore felt that matched controls must not be selected on the basis of perfect physical health.

(4) Drug and alcohol intake

All of the chronic schizophrenic patients were unmedicated with neuroleptic drugs at the time of study. Some of the acute patients (chapter 6) had been medicated with a variety of drugs prior to admission but the majority (and all those subjects studied in chapters 4,5 and 7 had not received neuroleptic drugs (as defined below).

In the case of the chronic schizophrenics the minimum accepted time since neuroleptic drug administration was one year and this was assessed by careful scrutiny of the medical and nursing notes. Several of the patients had never been exposed to neuroleptic drug at any time. This was accounted for by the presence in Shenley of a group of patients who had been admitted before the widespread use of neuroleptic therapy and who had subsequently been managed on a ward which employed psychotherapy and a token economy. Most of the patients had had brief or minimal exposure to neuroleptic medication usually many years in the past which had been discontinued due to either failure of therapeutic effect, side effects or non-cooperation. A smaller group of patients had been prescribed large doses of neuroleptics for many years which had been discontinued - also for the reasons given above.

In the case of acute schizophrenics the minimum drug free period for study was one month in the case of oral therapy and

six months in the case of depot neuroleptics. This was assessed by a combination of the accounts of the patient, their relatives and their general practioners. The unit in which these studies were performed has frequently intimated to the surrounding health care services an interest in schizophrenia and our willingness to see any suspected patients at very short notice. This service has undoubtedly ensured that a lot of patients have remained unmedicated who would normally have been placed on neuroleptics while awaiting outpatient appointments etc.

The ingestion of several other groups of drugs e.g. anti-depressants, anticonvulsants, lithium was also proscribed and no patients or controls had ingested these drugs within a month of testing. Benzodiazepine therapy and hypnotics were permitted but not within 12 hours of testing. All cases suspected of excess alcohol consumption or addiction were excluded.

A few of the schizophrenic subjects had been treated with a course of ECT and 2 had received modified insulin coma.

3.2 Clinical rating techniques

3.2.1 Mental State

This was assessed in terms of the standardised psychiatric assessment scale for rating psychotic patients devised by Krawiecka et al (1977). With this instrument the 8 variables - depression, anxiety, flattening and incongruity of affect, psychomotor retardation, hallucinations, delusions, incoherence of thought and poverty of speech/muteness - are assessed on a 0 - 4 point scale. In this study, flattening of affect and

incongruity of affect were rated separately to yield a total of 9 variables. Krawiecka et al provide a schedule describing how to elicit and how to rate these symptoms and some examples are given in Appendix C.

These ratings were carried out by two research psychiatrists experienced in the use of this schedule. In most cases these interviews were performed on the basis of a normal psychiatric interview. In the case of the apomorphine study (chapter 7) an interview constructed around the Krawiecka interview was video-taped and the recording subsequently rated by two psychiatrists independantly.

Delusions, hallucinations, incongruity of affect and incoherence of speech were taken together as 'positive symptoms' and flattening of affect and poverty of speech as 'negative symptoms' as described in Wing et al (1974). The maximum possible score on positive symptoms was 15 and for negative symptoms 8. The maximum score for positive symptoms is 15 because if a maximum score of 4 is rated for incoherence then only a maximum of 3 for delusions can be scored. This is because excessive incoherence precludes a full assessment of psychotic features such as delusions which require clear description. The non-specific features of retardation, depression and anxiety were recorded. No subject exhibited depression to a morbid degree.

3.2.2 Cognitive functioning

This was assessed in terms of the Withers and Hinton (1971) series of tests of the sensorium. Previous experience with this type of population showed this test to be satisfactory in that

most patients are able to attempt it and achieve some score.

No testing of control populations was undertaken for the present study but several control groups have previously been studied by the investigators who performed the ratings for the present study (Johnstone et al, 1978). Control groups matched for age and educational background with chronic schizophrenic patients and a group of physically disabled subjects have been studied and the mean score for these groups (± SD) is 80 ± 20.

A few more specific measurements were made (e.g. blink rates) which will be described in the appropriate sections.

3.3 Sample collection, handling and storage

3.3.1 Venous sampling techniques

Venepuncture was performed in the standard way from antecubital veins.

Serial venous samples were obtained by the following method:- a 21 gauge butterfly needle attached by a short length (18 cm) of plastic tubing to a rubber sampling bung was inserted and taped into a forearm vein below the cubital fossa. Samples (approx. 5 ml) were withdrawn by syringe and needle from the rubber bung and the cannula flushed by the injection of 1 ml dilute heparinised saline solution (2 ml 1000 units preservative-free heparin/ml with 10 ml saline). Prior to venous sampling 2ml was first withdrawn and discraded to minimise saline dilution and heparin contamination of the sample. These butterfly cannula were very successful both in terms of not restricting patients' movement and activity and in terms of remaining patent for venous sampling. In general it was noted that the vein became

somewhat tender about 2 hours after insertion but this did not prove a major restriction. The actual insertion of the cannula was marginally more painful than direct venupuncture and usually a period of 15 - 30 min was left prior to taking the first test sample.

3.3.2 Handling and storage of samples

Blood was immediately transferred to a plastic container and placed in a 4 ^{o}C fridge. After 24 hours the samples were centrifuged at 3 x 10^{3} rpm for 15 min in a bench centrifuge. Serum was pipetted off and dispensed into .5 - 1.0 ml aliquots in plastic containers. These were then transferred immediately to a liquid nitrogen store (-170 ^{o}C) where they were stored until assay. When samples were assayed they were allowed to thaw slowly. After assay the samples were snap frozen by plunging them into liquid nitrogen. Subsequently the samples were stored at -40 ^{o}C. Since several aliquots had been made at the time of the original preparation of sera and the number of assays performed relatively limited the number of times that each sample was thawed and frozen was never more than two.

3.4 Radioimmunoassay (RIA) techniques

Nine different hormones were measured in sera from schizophrenics and controls. LH, FSH, GH, PRL, TSH and testosterone were measured in the CRC, by previously published methods (see Table 3.1), by the author. T3 and T4 were measured by standard RIA techniques by Miss S. Copping in the Division of Endocrinology Research, CRC. Oestradiol was measured at the WHO Collaborating

Centre for the measurement of reproductive hormones, Chelsea Hospital for Women, London, SW3 by Dr. I. Rincon-Rodriguez.

An outline of the reagents and methods of each of these assays is given in Table 3.1 and a brief description of theory and practice is given below. The iodinated tracer for assay of the LH, FSH, PRL and GH was prepared by the author and methods for purification and evaluation of this material developed. These procedures are outlined below and amplified in Appendix D. Finally descriptions of the methods of separation of bound and free label, containing radioactivity and quality control procedures are discussed.

3.4.1 Radioimmunoassay - theory

The basic theory of RIA is depicted in Fig 3.1. The basis of the assay procedure is the competition for binding between specific antibody and isotopically labelled antigen (Ag) and unlabelled Ag. In the presence of large amounts of unlabelled ('cold') Ag in a sample, the amount of labelled antigen bound to antibody is reduced by displacement. A suitable method for separating bound and free Ag must be devised. Standard displacement curves are derived using known concentrations of the Ag (in this case a hormone) and the amount of displacement produced by an unknown concentration can then be measured and its equivalence in hormonal units determined.

A number of aspects of assays must be considered. The concentration of labelled antigen and antibody are carefully adjusted to give optimum binding within the range of expected hormone levels. To increase sensitivity of the assay a pre-incubation of

FIG 4.1

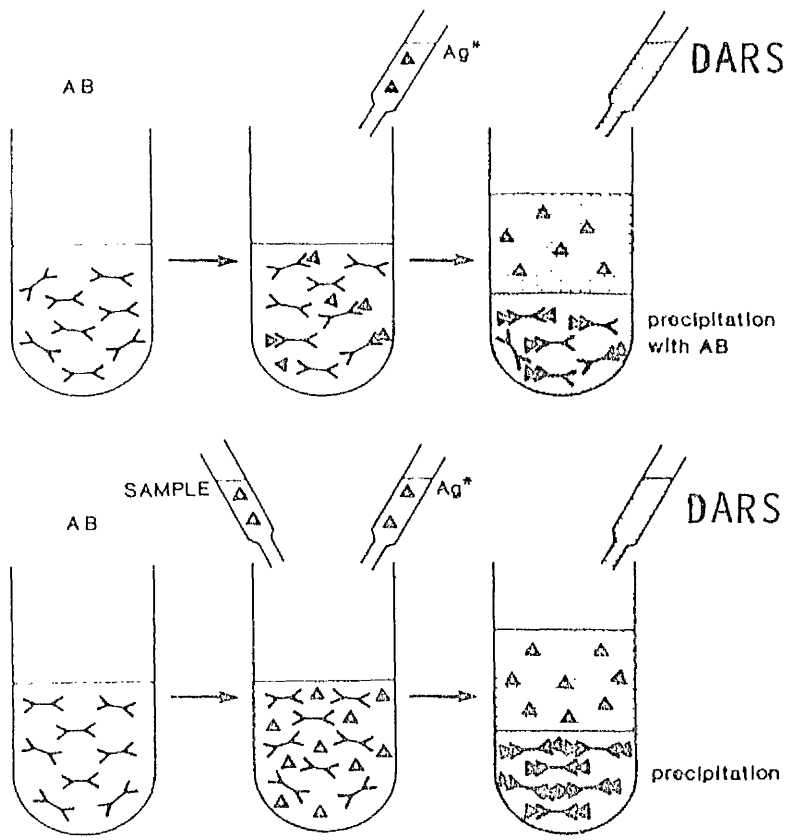


Diagram of principles of radioimmunoassay. Upper part shows addition of Ag* to antibody (Ab) and precipitation of the labelled hormone with donkey anti-rabbit serum (DARS). In lower part, addition of unknown sample results in competition with Ag* for AB. The reduction in Ag* bound to AB results in fewer counts in the precipitate.

sample/standard and antiserum prior to addition of labelled antigen was carried out. If a sample proves to be above the level of the sensitivity of the assay then it must be diluted with buffer or other solutions till its level is within the range of the assay. Before this can be performed with confidence the parallelism of the assay needs to be checked: that is that the assay system reacts to naturally occurring hormone diluted serially in the same way as the standard hormone. The parallelism of all assays used in the present studies has been fully checked and is satisfactory (Cotes et al, unpublished). Details are also available in the original methodological descriptions cited in Table 3.1.

The antibodies used in a RIA immunoassay must be specific and the labelled Ag virtually pure. Cross-reactivities with a variety of possible interfering substances for each of these assays have been checked and were invariably less than 1%. It must be emphasized that RIA measures immunological and not biological activity. A detailed description of the precise structure of the hormones studied here and the extent to which the antibodies used react with either a pro-hormone or subunits of the hormone is beyond the scope of this work.

3.4.2 RIA - materials used

a) Buffers were made up as indicated in Table 3.1 using the purest reagents available (Analar), pH and conductivity of buffers were checked on each occasion on a Philips Digital pH meter and a Radiometer (CDC 104) respectively.

b) Assays were performed in 2 ml Sardstedt polypropylene

tubes which were vortexed mixed after each addition of reagent.

c) Samples were pipetted into tubes using a "Compupette" (General Diagnostics, N.J., USA). This machine allows for the dispensation of 10 - 2000 µl of fluid. It is highly reliable (the variation was frequently checked and found to be of the order of ± 1%) but was less accurate (± 5%).

d) Standards were stored in freeze-dried preparations and a range for assay was made up by serial dilutions on each occasion of assay.

e) Antisera and iodinated antigen were also stored at -40 ^{o}C prior to use.

f) Methods were otherwise as described in original papers, and the following sections (3,4,5,6,7) and appendices.

3.4.3 RIA - individual assays

All samples and standards were assayed in duplicate.

1) LH. The LH assay was carried out according to the protocol in Table 3.1 by the method described in Cotes et al (1978). Results are expressed in U/L according to 1st International Reference Preparation (IRP) of human LH for immunoassay (MRC 68/40). The mean range of sensitivity of the assay (see section 3.4.6 for this definition) was 2.0 - 50 U/L. An example of a typical LH standard curve is discussed in Appendix D.

2) FSH. This was carried out according to the protocol in Table 3.1 and the method of Cotes et al (1978). Results are expressed in U/L in terms of the IRP of human FSH and LH for bioassay (MRC 69/104). The sensitivity of the assay was 0.5 - 50 U/L.

TABLE 3.1

Hormone	Buffer	Antigen and label	Standard	Antiserum concentration	Assay protocol (hrs)			Sensitivity	Method reference
					Pre-Incubation	Incubation	Separation		
LH	0.05M PO_4 0.01M EDTA	LH 2/3 ^{125}I	IRP Human LH (68/40)	anti-human F87 1:2,000,000	6	24	DARS/NRS overnight (see text)	1.5-100 iU/L	Cotes et al, 1978
FSH	0.15M NaCl 0.01% Thio 0.5% BSA	FSH CPDS 1B ^{125}I	Human pituitary FSH and LH (69/104)	anti-FSH M93 1:3,000,000	6	24	"	0.5-50 iU/L	"
GH	0.05M PO_4 0.01% NN_3 2% Horse Serum	HGH 69/46 ^{125}I	2nd UK HGH Working Standard	anti-HGH 66 1:200,000	6	24	"	0.6-12 mU/L	"
PRL	0.01M PO_4/EDTA 0.15M NaCl 1% HSA	HPRL (Lowry) ^{125}I	IRP Human PRL for Immunoassay (75/504)	AFP anti-human PRL 1:200,000	72	48	"	50-800 iU/L	Modified from Cotes et al, 1978
TSH	0.07M PO_4 0.15M NaCl 2% Albumin 20 iu HCG/ml	Human TSH DE-52-5 ^{125}I	WHO 1st IRP Human TSH (69/58)	anti-human TSH (72/554) 1:200,000	72	24	"	2-25 mU/L	Byfield et al, 1979
Testosterone	0.02M PO_4 0.15M NaCl	Testosterone ^3H	Testosterone (Sigma)	anti-testosterone -3 BSA 1:4000	-	24	Charcoal (see text)	5-100 nmol/l	Modified from Collins et al, 1972
Oestradiol	0.01% Thio 0.1% gelatin	17βoestradiol ^{125}I	17βoestradiol (Sigma)	anti-17βoestradiol 1:40,000	-	24	"	10-200 pmol/l	Modified from Emment et al, 1972

Details of assay protocols used in studies showing buffer, antigen, standard, antiserum and concentration used together with details of incubation period, separation methods and basic published reference. Further details are given in the text.

3) GH. This was measured in outline as described above. Results are expressed in mU/L in terms of the 2nd UK Working Standard and the test range of the assay 0.6 - 12 mU/L.

4) PRL was measured according to the protocol set out in Table 7.1. This method is as set out in Cotes et al, 1978 except that the peptide for iodination was modified from the VLSpeptide to the purer HPRL (Lowry). The standard for PRL was the International Reference Preparation of Human Prolactin for Immunoassay (75/504) and the sensitivity of this assay lay between 50 - 800 IU/L.

5) TSH was measured by RIA according to the method of Byfield et al (1979). The standard was the WHO 1st International Reference of Human TSH for Immunoassay (MRC 68/38) and the sensitivity of this assay was 2.0 - 25 mU/L.

6) T4 and T3. Thyroxine and tri-iodothyronine were measured by a semi-automated radioimmunoassay in routine use at Northwick Park Hospital. The interassay co-efficients were respectively 7% and 5%. The normal adult ranges for the T4 assay is 60 - 150 nmol/l and 1.2 - 2.8 nmol/l for the T3 assay.

7) Testosterone. Samples for testosterone assay were first extracted in pure ethyl alcohol which was evaporated with liquid nitrogen. The method is described in the WHO Collaborating Centre Manual (1981). Standard was supplied as 10 ng/ml testosterone and diluted serially. Separation was carried out by the addition of ice cold charcoal-dextran (see below).

8) Oestradiol. Samples were extracted as documented above for testosterone. The method is described in the WHO Colloborating

Centre Manual (1981). The method used was a modification of the method of Emment et al (1972) so that ^{3}H-oestradiol was replaced by ^{125}I-oestradiol.

3.4.4 RIA - Iodination of peptides

The following antigens were labelled with I^{125} by the author:- FSH, LH, GH and PRL. The antigen used for each is delineated in Table 3.1. A chloramine-T method was used in the case of FSH, LH and GH: PRL was iodinated by the lactoperoxidase method. The methods used are fully described in Hunter (1982) but an outline is given in Appendix D where some specific examples are also shown.

3.4.5 RIA - Separation techniques

An adequate method of separating bound from free labelled antigen is essential in any RIA system. The methods employed in the assays of the present studies are outlined in Table 3.1. As can be seen these were broadly of two types: a) addition of a second antibody directed to antibody or b) the addition of charcoal.

The second antibody employed in the current study was donkey anti-rabbit sterum (DARS) raised against rabbit IgG (the antibodies used in the RIA system were raised in rabbits). Thus antibody would be precipitated and where this was linked to labelled antigen counts would be measurable. To add stability to precipitates a small amount of normal rabbit serum (NRS) was added. The concentrations of DARS and NRS were carefully adjusted to obtain maximum precipitation of antibody with maximum stability of the precipitate and with good safety margins. It was

demonstrated that the optimal concentrations were 4 µl DARS/tube and 0.25 µl NRS/tube and these concentrations were used throughout. It was shown that separation could be satisfactorily be performed 8 hours after the addition of DARS but in practice separation was performed after overnight incubation with DARS/NRS (approx. 16 hours). At this point 1 ml of buffer with a trace of detergent (Triton 100 - final concentration .2%) was added - the so called "wash". This additional volume ensured that when the supernatant was discarded it was relatively dilute and so if traces of fluid could not be displaced then their contribution in terms of radioactivity would be minimal. The addition of detergent also reduced the surface tension of the supernatant to minimise retention when inverted. The samples were then centrifuged at 2.5×10^3 rpm in a refrigerated centrifuge for 45 - 60 minutes. The tubes were then rapidly inverted over a pronged rack so that the supernatant was discarded. The tubes were then left to drain for 15 min prior to counting.

Charcoal

Norit A Charcoal (Sigma) was dissolved in phosphate buffer with an equal weight of dextran 70. The separation is performed at 4 °C. 1 ml of dextran coated charcoal (maintained in suspension by a magnetic stirrer) was added quickly to all tubes. The contents were mixed on a rota mixer, allowed to stand for 15 min and centrifuged (4 °C, 2000 g, 15 min). The dextran coated charcoal adhers to the bottom of the tube and the supernatant containing the bound fraction was decanted into vials containing 10 ml of counting fluid.

3.4.6 RIA - Techniques of counting radioactivity

After the supernatants were decanted, the precipitants were allowed to dry and the activity of I^{125} counted on a NE1600 scintillation counter (Nuclear Enterprises, Reading, UK) with an efficiency of approx. 60% in batches of 16 samples. Counts were continued long enough for trace (no unlabelled antigen) binding tubes to reach approximately 10,000 counts (usually 200 - 300 seconds). Standards and samples were counted for the same length of time immediately consecutively. Background counts were repeatedly checked and for the range of counting times cited above were below 200 counts. No background subtraction was made. A paper tape was made which could be read directly into a computer file.

The scintillation fluid used for the testosterone assay consisted of 2 volumes toluene, 1 volume of Triton X-100 and 4 g PPO per litre of mixture. Before counting samples were allowed to equilibrate for at least an hour. Samples were counted on a Wallach counter (LKB, Sweden).

3.4.7 RIA - Computer analysis of raw data

Potency estimates were derived from observations of radioactivity counts bound, using a computer program written by Dr. R. Wootton based on Healy's (1972) 4-parameter log-dose logit-response standard curve. An example of the type of curve generated is discussed in Appendix D. Samples with binding greater than 85% and less than 15% trace binding (no unlabelled antigen) were deemed outside the range of sensitivity of the assay (see Appendix D for further details).

This computer program has been checked against various other methods of calculating estimates at the National Institute of Biological Standard and Control. The program rejected poor duplicates depending on the magnitude of their discrepancy and their position on the dose-response curve.

3.4.8 RIA - Quality control

It is increasingly being recognized that it is very important to set up a system of quality control for RIA whereby the accuracy and the variability of assays can be checked. These two measures can vary independently i.e. an accurate assay may give very variable results from assay to assay: alternatively a reliable assay may produce very "tight" data but generate data nowhere near the 'true' result. For each of the assays performed here at the CRC (assays 1 - 7, section 4.3) the following protocol for establishing inter and intra assay variation was performed.

Samples were obtained which contained high (near the upper limit of assay detection), medium and low (near the lower limit of detection) concentrations of the hormone under test. These samples were obtained from a variety of sources (e.g. high FSH and LH samples were obtained from post menopausal females, high TSH samples from patients with hypothyroidism, high GH sample from a control subject taking 'Bovril', high PRL sample from a patient on neuroleptics etc.). Various sera were then mixed to reach approximate concentrations. These samples were then aliquoted, so that an aliquot could be measured in every assay. The inter assay variation was then calculated by finding the mean

and SD of these estimates and dividing the SD by the mean (co-efficient of variation = SD/mean X 100%). Assays were only accepted when the estimates for each of the high, medium and low quality controls (QC's) fell within the mean ± the co-efficient of variation. If assays were within this range then values estimated were accepted and no correction factors were applied. Intra-assay variation was checked by repeated random estimations of a sample (whose level was close to the expected levels of the normal population) within one assay. Details of the QC's used for each assay and the calculated inter and intra assay variations over all the assays performed are given in Appendix D. In practice, a series of assays were undertaken for one particular study and the assay variations for the series are given in the methods section of each separate study.

To minimise the effects of inter assay variation, each assay contained all the samples from one patient on one particular test protocol and each assay contained samples from patients and controls.

CHAPTER 4

Basal pituitary hormone levels in acute and chronic schizophrenia, with particular reference to gonadotrophin and prolactin hormonal rhythms

INTRODUCTION

As discussed in chapter 2 there is increasing interest in the study of pituitary hormone secretion in psychiatric illness. This is largely based on the hope that thereby diagnostic markers may be developed and an understanding of the aetiopathological basis made. This section reports on hormonal estimates from acute and chronic schizophrenics, diagnosed according to strict criteria. Particular reference is paid to hormonal rhythms since dynamic aspects of hormonal secretion have important underlying control mechanisms and single estimates of hormones are methodologically problematical.

As discussed in chapter 1 (section 2.5) and chapter 2 (section 2.6) 3 groups of workers have demonstrated low gonadotrophin secretion in patients with chronic schizophrenia, although the frequency of these abnormalities varied from 50% of cases (Shader et al, 1968; Johnstone et al, 1977) to 100% (Brambilla et al, 1975). Gonadotrophin secretion in acute schizophrenic patients has scarcely been studied. It has become recognized that the secretion of LH is episodic. There are bursts of LH secretion occurring at irregular intervals throughout the 24 hours (Boyar et al, 1972). There are 12 - 20 of these episodes per day (defined as an increment greater than 30% of the preceeding level) (Santen and Bardin, 1973). FSH exhibits a similar

though less marked pattern. In this study LH and FSH were measured serially in patients with chronic schizophrenia in order to a) examine the incidence of gonadotrophin secretion abnormalities in a population of chronic schizophrenics b) determine whether the normal episodic secretory pattern of gonadotrophin secretion is disturbed in schizophrenia and the degree of reliability of various measures of this and c) assess the relationship between gonadotrophin secretion and various clinical characteristics of the patients studied.

In addition, the following hormones - PRL, GH, T3, T4, TSH, testosterone and oestradiol were measured in schizophrenic patients and the reasons for the inclusion of each of these hormones are set out below.

PRL and GH secretion are of considerable theoretical importance in neuroendocrine studies of schizophrenic patients, since DA is important in the regulation of both these hormones. Several studies reviewed in chapter 2 (section 2.6) have indicated that the basal levels of these hormones are normal in both acute and chronic schizophrenia. However these hormones have characteristic neural-regulated rhythms of secretion (detailed in chapter 2 (section 1.3)) and these rhythms have not been studied in schizophrenic patients.

Several studies have indicated that thyroid function (e.g. Kline et al, 1968) (including TSH secretion (Johnstone et al, 1977)) is normal in schizophrenia. The present study provided an opportunity to replicate and extend such work. Abnormalities of thyroid function can alter hormonal responsivity to various

agents (e.g. drugs and hypothalamic releasing factors) and so thyroid function estimations are important prior to embarking on studies which employ such provocative agents (chapter 5, 6 and 7).

Testosterone was measured in schizophrenic patients in an attempt to resolve some outstanding problems raised by previously published studies. These are:- 1) Brambilla et al (1975) reported a large reduction in serum testosterone in all of the chronic schizophrenic patients they studied. This has not subsequently been replicated. Moreover urinary testosterone has been found to be reduced only by a small amount and only in a sub-group of chronic schizophrenics (Brooksbank et al, 1970) 2) Beumont et al (1974,a) reported that serum testosterone levels rose (into the normal range) in chronic schizophrenics after neuroleptic therapy was withdrawn. These studies indicate that the large reductions found in testosterone by Brambilla et al (1975) may have been due to the short time following neuroleptic withdrawl prior to study.

This study was also used as an opportunity to measure serum oestradiol levels in schizophrenic patients. This measure is of particular interest because levels of oestrogens have been shown to influence both LH secretory rhythms (Santen and Bardin, 1973) and PRL secretion (Martin et al, 1977) both of which were assessed in the present study. In addition there are strands of evidence from older studies that urinary secretion of oestrogens is increased in schizophrenic subjects (Hoskins and Pincus, 1949; Sands, 1957) which have not been replicated using more modern methodology.

SUBJECTS, MATERIALS AND METHODS

Subjects

Five groups of subjects were studied as outlined below. Clinical characteristics are given in Table 4.1.

1) Chronic schizophrenic group (CS)

Twenty long term inpatients were selected from a large sample of schizophrenic patients who conformed to criteria of Feighner et al (1972) (see chapter 3.1.1 and Appendix A). The large sample from which the group studied here was drawn have been documented in detail elsewhere (Owens and Johnstone, 1980). Some results from that survey were presented in chapter 1. The 20 patients studied here were selected on the basis that they

1) were male
2) were aged less than 70 yrs
3) had been off neuroleptic medication for at least a year prior to the study (see chapter 2 for further details of the analysis of drug history and Table 4.1 for specific details for this group) and
4) were agreeable to inclusion (see chapter 2 for further details).

These patients exhibited normally developed secondary sexual characteristics. Because of the nature of their mental state and life in a large psychiatric hospital it was impossible to assess libido or estimate the frequency or type of sexual experience. Physical examination did not reveal any significant medical abnormality other than arthritis (probably osteoarthritis) and mild hypertension (1 case each). As can be seen from Table

TABLE 4.1

Group/Code	Diagnosis	No. and sex	Age (yrs)	Weight (kg)	Length of sampling (h)	No. of samples	Age at 1st hospitalisation	Positive symptoms*	Negative symptoms*	Drug History
1/CS	Chronic schizophrenia	20 M	58 ± 8 (38-69)	66 ± 15 (41-104)	3.1 (2.5-4.5)	9 (7-15)	26 ± 5 (17-40)	2 (0-12)	4 (0-7)	Stopped neuroleptics 1-12 yrs previously (mean 4-5) 5 never received neuroleptics
2/CONT	Controls (see text)	17 M	60 ± 7 (45-70)	75 ± 12 (59-101)	2.9 (2.0-3.5)	8 (7-10)	-	-	-	Off antidepressants for 1/12 - 5 yrs. 4 on Benzodiazepines
3/AS (remission)	Acute schizophrenia (remission)	3 M	25 ± 6 (18-32)	70 ± 12 (58-82)	2.4 (2.0-3.0)	9 (8-10)	22 ± 6 (16-28)	0	1 (0-1)	Stopped neuroleptics 9 - 18/12 prior to study
4/AS	Acute schizophrenia	30 M	28 ± 2 (20-49)	-	-	1	26 ± 2 (16-49)	9 (1-15)	1 (0-5)	18 never received neuroleptics 12 stopped neuroleptics at least 1/12 before (mean 9/12)
5/RP	Controls (see text)	30 M	59 ± 8 (38-71)	-	-	1	-	-	-	2 on β Blockers 4 on Diuretics 4 on Antacids
	Normal volunteers	11 M	28 ± 3 (22-35)	-	-	1	-	-	-	None

Clinical data of patients reported in this chapter along with details of sampling schedules from those patients tested with serial sampling. Values as means ± SEM except symptoms * - values as modes.

4.1, the weight of this group was less than their age-matched control group (2 below) but this difference is not significant.

On a subsequent occasion some 3 - 9 months later eleven of these patients were retested in a similar fashion to that described below. The clinical state and drug free status of these patients was identical on the second occasion of testing.

2) Controls (CONT)

Seventeen subjects were studied as controls (Table 4.1). This group was comprised of several subgroups as follows:-

a) 3 normal healthy volunteers

b) 3 were long stay patients of the same hospital as the CS patients (group 1, above) with a diagnosis of manic depressive psychoses on the Feighner criteria (see Appendix A). At the time of the study none of these patients showed evidence of affective disturbance.

c) 5 men attending outpatients with a diagnosis of mild reactive depression (Using the criteria of Feighner et al (1972) these patients fell into the cateogy of "unspecified psychiatric disorder - category 1" and all were symptom free at the time of study).

d) 6 were patients recently admitted to an orthopaedic ward for elective surgery for non-metabolic disease (osteoarthritis (2), ganglion (1), Dupytren's contracture (2), removal of pin (1)).

None of the above subjects were taking psychoactive medication at the time of study. Other drugs ingested by this group are documented in Table 4.1. There was no evidence that any subject

in this (or indeed in any) group abused alcohol or drugs.

3) Remission from acute schizophrenia

Three patients who had previously been admitted with acute nuclear schizophrenia (NS + on Catego program of the PSE - see chapter 2) were studied. These patients had been admitted, with their first episode of schizophrenia 19 - 36 months previously. Mental state was normal (see below) at the time of study. All were gainfully employed. They had discontinued neuroleptic medication of their own accord 6 - 9 months prior to this study (2 have subsequently relapsed).

In addition single venous samples were taken from:-

4) Acute schizophrenia

30 male patients recently admitted with acute schizophrenia (see section 1.2, chapter 3 for the definition of this classification) were studied. These patients were also the subjects of the studies described in chapters 6 and 7. All patients were drug-free at the time of study (see section 1.4, chapter 3 for definition of drug-free for this group). Further clinical details are furnished in Table 4.1.

5) Reference Populations

Single venous samples were obtained from two groups of volunteer subjects selected to age match groups (1) and groups (3) / (4) respectively (Table 4.1). These were respectively a) 30 men - 15 normal healthy normal volunteers and 15 attending outpatients with non-endocrine disease (e.g. peptic ulceration, chronic bronchitis, minor orthopaedic problems) and b) 11 normal male healthy medical and paramedical hospital staff.

Methods

Venous sampling

All subjects from groups 1, 2 and 3 were studied under similar circumstances. After an overnight fast a butterfly cannula was inserted at 8.00 a.m. and kept open with dilute heparanised saline which was discarded prior to sampling (further details are given in chapter 3.3.3). Blood samples were taken at 15 - 20 minute intervals for 2.5 - 4 hours. Sampling periods were comparable in both groups (see table 4.1 for details). At 0900 hours a light breakfast was given.

Single samples were obtained by venupuncture from subjects in groups 4 and 5. Samples from acute schizophrenics (group 4) and their age-matched control group (5, b) were taken fasting at 0830 hrs. Samples from group 5a) (older reference group) were taken between 0930 and 1030 hrs as they were control samples for the mean levels of the CS group (1).

Sera was prepared as outlined in chapter 3.3.3.

Radio-immunoassay

The following hormones were estimated in samples from these groups LH, FSH, PRL, GH, TSH, T3, T4, testosterone and oestradiol. A full description of the methodology of each of these assays was given in Chapter 3 (section 4.3). For estimates of each substance, all the samples from each patient were estimated in a single assay. All assays contained samples from patients and controls in a balanced design. Theoretical aspects of quality control protocols were discussed in Chapter 3 (section 4.8). Specific details of the number of assays performed and the intra

and inter-assay variation for a range of quality controls is given in Table 4.2.

Serial estimations of LH, FSH, PRL and GH were made for groups 1, 2 and 3 whereas T3, T4, TSH, testosterone and oestradiol were measured in single samples only in all of the groups. In the case of LH and FSH, a mean value has been calculated for the series of estimates and the co-efficient of variation (CV%) was computed by dividing the standard deviation (SD) by the mean (CV% = SD/mean X 100%).

Mental State Mental state of the schizophrenic patients was assessed on a 0 - 4 scale according to the method of Krawiecka et al (1977) which is described in detail in chapter 3 (section 2.1). Cognitive function was assessed in the CS group by the Withers and Hinton (1972) test of the sensorium (see chapter 3.2.2).

Statistics In relating clinical data to hormonal estimates in serum a non-parametric analysis (Spearman's rank correlation test) was performed. Comparisons between groups were made using the Student's t-test (two tailed) unless otherwise stated. An examination of LH and FSH data revealed a skewed distribution. Plots of LH and FSH indicated that the transformation $X = \sqrt{1/X}$ was adequate to make the data normally distributed. Comparisons between groups in terms of LH and FSH were analysed by one way analysis of co-variance. Parametric correlations were performed on untransformed data.

Results

Levels of hormones estimated in all of the groups are set

TABLE 4.2

Hormone	No. of Assays		Quality Control Data				Intrassay variation
			QC_1	QC_2	QC_3	QC_4	
LH IU/l	15	Mean SD CV%	50.3 7.6 15.1	11.4 0.5 4.6	10.7 1.2 10.9	6.8 1.0 14.6	5%
FSH U/l	8	Mean SD CV%	5.7 0.6 10.5	3.7 0.3 8.1	2.2 0.1 6.8	-	5%
GH mIU/l	12	Mean SD CV%	10.1 0.9 8.9	3.7 0.2 5.8	1.6 0.2 12.5	0.8 0.2 17.5	3%
PRL mIU/l	12	Mean SD CV%	738 86 11.6	411 48 11.7	167 13 7.6	-	4%
TSH mU/l	5	Mean SD CV%	2.0 0.2 10.0	20.4 2.2 10.8	-	-	6%
Testosterone n mol/l	4	Mean SD CV%	21.4 3.6 16.8	44.6 4.9 11.0	-	-	10%

Quality control data for assays reported in chapter 4. Intrassay variation is small for each of the assays. Co-efficiencts of variation are of the order of 10% in the middle of the assay range but somewhat larger towards the limit of assay sensitivity (see chapter 3 (4.8) and appendix D for further details)

out in Table 4.3. Values for LH, FSH, PRL and GH are the means of mean values for groups 1, 2 and 3 but are from single samples for the remaining hormones and for all estimates from groups 4 and 5.

a) Comparison of results between CS group (1) and their age-matched control group (groups 2 and 5a)

There was no difference between the CS group and each of the control groups or both combined in thyroid function (TSH, T3, T4), gonadal steroids (testosterone and oestradiol) or PRL and GH secretion. LH and FSH secretion were significantly reduced in the CS group compared to either control group and both groups combined (see Table 4.3 for significance levels). An effect of age on LH and FSH secretion was noted in both groups (see below) and therefore a one way analysis of co-variance was carried out allowing for this effect. Using this analysis LH and FSH were significantly reduced below the control group ($F(1,62) = 4.57$, $p < 0.05$: $F(1,62) = 11.25$, $p < 0.001$ respectively).

b) Comparison of results between acute schizophrenics in remission (group 3), acute schizophrenics (group 4) and age-matched control group (group 5b)

As can be seen from Table 4.3 there were no differences in LH, FSH, GH, PRL or oestradiol secretion between these groups.

c) Results of serial sampling (Figs 4.1, 4.2, 4.3)

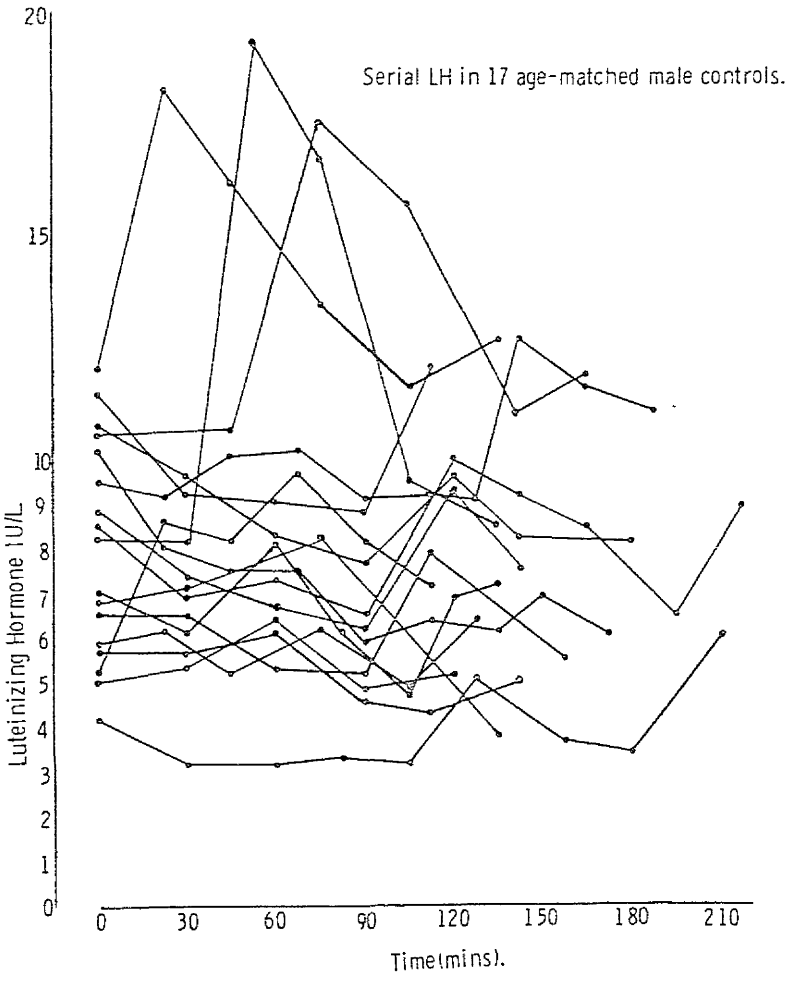
1) LH and FSH. The co-efficient of variation (CV%) from all estimates of LH and FSH was calculated as described above for each subject for whom serial samples were obtained; the mean

TABLE 4.3

Group	LH iu/l	FSH U/l	GH mU/l	PRL miU/l	TSH mU/l	T3 n mol/l	T4 n mol/l	Testosterone n mol/l	Oestradiol p mol/l
1. Chronic schizophrenics n = 20	6.2 ±0.6	2.7 ±0.3	1.6 ±0.3	150 ±16	3.5 ±0.5	2.0 ±0.1	79 ±3.0	27.4 ±2.7	71.1 ±5.9
2. Controls n = 17	9.1* ±1.1	7.4* ±3.2	0.9 ±0.1	155 ±24	2.9 ±0.3 (9)	1.9 ±0.1 (9)	70.7 ±4.5 (9)	25.1 ±2.4	70.3 ±7.0
3. Remission from acute schizophrenia n = 3	5.5 ±0.3	1.7 ±0.3	-	169 ±26	-	-	-	-	-
4. Acute schizophrenics n = 30	7.6 ±0.8	2.1 ±0.2	1.4 ±0.2 (15)	225 ±23	-	-	-	-	52.1 ±7.3 (10)
5. Reference Populations a) Older group n = 30	9.2** ±1.3	6.3** ±1.5	0.9 ±0.2	159 ±15	-	2.2 ±0.1 (13)	79.8 ±2.8 (13)	26.4 ±1.7 (13)	76.8 ±9.0 (13)
b) Younger group n = 11	6.1 ±0.5	2.4 ±0.5	1.1 ±0.3 (10)	234 ±23	-	-	-	-	60.1 ±9.5 (10)

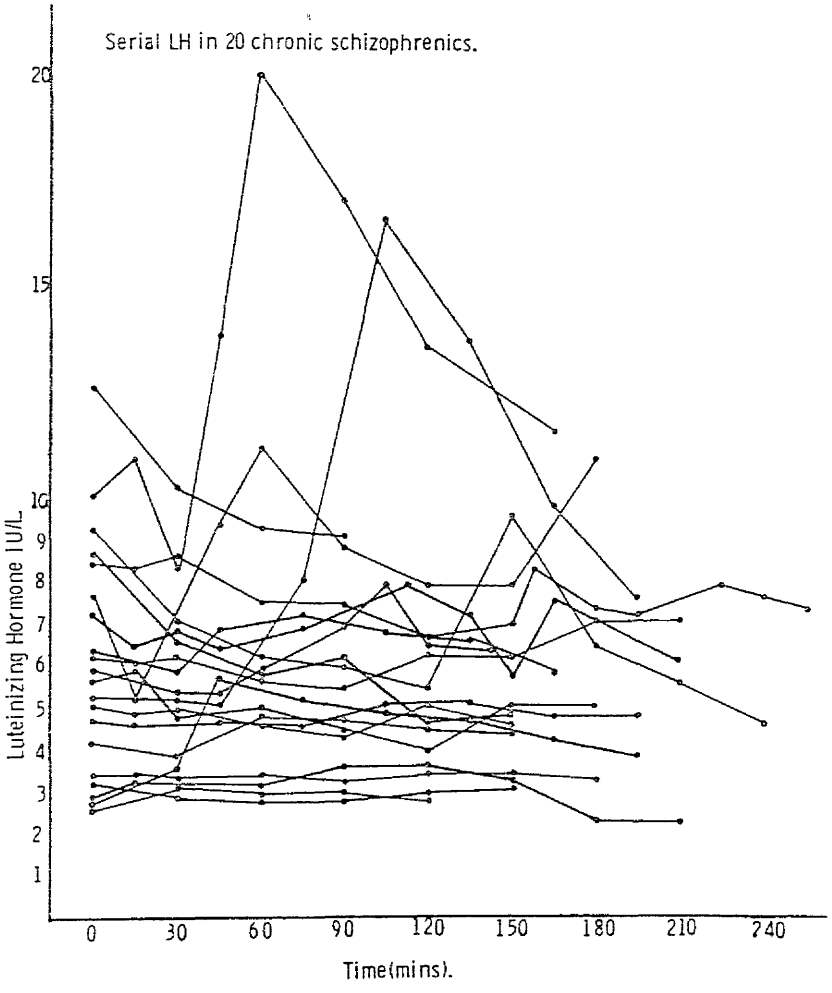
Basal levels of hormones in 5 groups studied (see text for details). * $p < 0.05$ ** $p < 0.01$ compared with chronic schizophrenics. No other significant differences. Number studied in parentheses.

Fig. 4.1



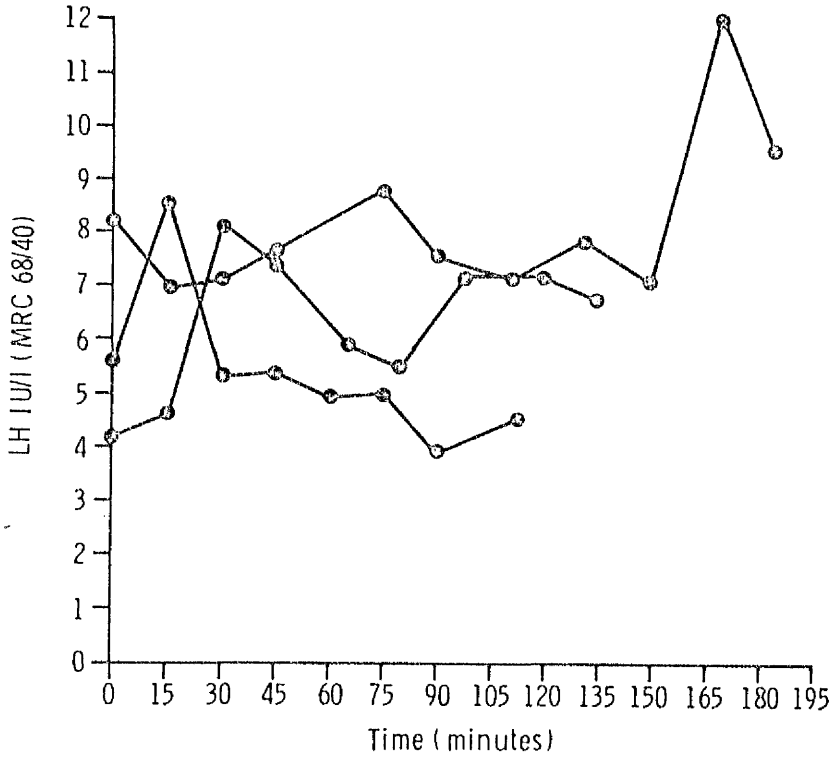
Serial LH estimations every 20 minutes in samples from 17 elderly men.

Fig. 4.2



Serial LH estimation every 20 minutes in samples from 20 chronic schizophrenics.

Fig. 4.3



Serial LH levels in 3 acute schizophrenics in remission. All exhibit evidence of episodic secretion.

TABLE 4.4

		LH (IU/L			FSH U/L			Number of patients with evidence of a LH increment greater than 50% of baseline	Mean number of positive LH increments per hour of sampling
		Mean	SD	CV%	Mean	SD	CV%		
1) CS n=20	Mean	6.2	0.9	14.8	2.7	0.4	14.2	7 (35%)	0.12
	SD	2.7	1.0	11.1	0.3	0.3	5.0		
2) CONT n=17	Mean	9.1*	1.7**	18.6*	7.7**	0.9*	12.8	12 (71%)	0.39***
	SD	4.5	1.1	6.6	13.0	0.3	7.4		
3) AS n=3	Mean	5.5	1.0	18.0	1.7	-	-	3(100%)	0.56***
	SD	0.9	0.3	5.3	0.6				

LH, FSH and the co-efficient of variation for serial estimates of LH and FSH (CV%) in groups studied (means ± SEM) : percentage of patients with and frequency of positive LH increments.
* $p < 0.5$ ** $p < 0.01$ *** $p < 0.001$.

values for each group, together with the mean serum level for the group, are shown in Table 4.4. Previous workers (Boyar et al, 1972; Santen and Bardin, 1973) have defined an LH secretory episode as a positive increment of 30% above the previous level. The number of subjects in each group whose serial sample estimates gave evidence of this phenomonen is shown in Table 4.4. The mean number of such episodes per hour for each group is also shown in Table 4.4

The mean level of LH and FSH is lower in the schizophrenic group than in the CONT group. The coefficient of variation is lower in the CS group compared with the CONT group for LH ($p < 0.05$, standard Student's t-test) but not for FSH.

There was a cluster of CS patients ($n = 10$) in whom the coeficient of variation for LH is less than 10% (twice the within-assay coefficient of variation), whereas none of the controls exhibited such low levels of fluctuation in serial LH estimates (Figs 4.1, 4.2).

According to the criteria set out above for defining an LH secretory episode, 12 of the 17 controls (71%), but only 7 out of the 20 chronic schizophrenics (35%), exhibited evidence of episodic LH secretion. All of the young schizophrenic patients had evidence of episodic LH secretion (see fig 4.3). An episode of LH secretion tended to be followed by the characteristic decrement in the LH level to baseline.

As shown in Table 4.4, the frequency of LH episodes per hour is much less in the CS group (0.12 episodes per hour or 1 episode every 8.3 hours) than in the CONT group (0.39 episodes

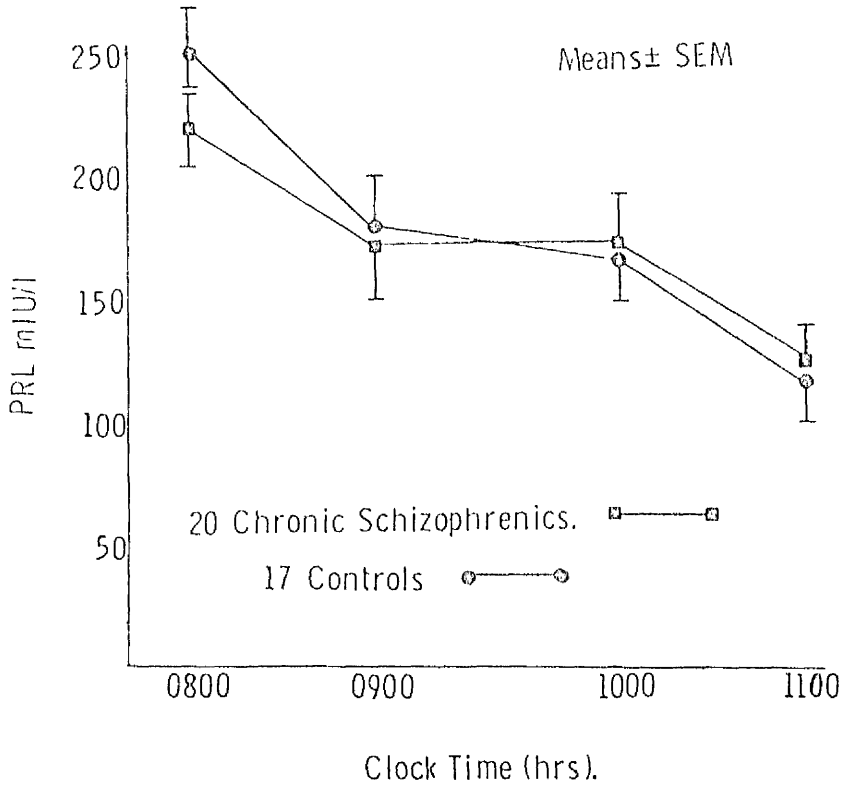
per hour or 1 episode every 2.6 hours) and this difference was statistically significant at the $p < 0.005$ level. The mean frequency of LH secretory episodes in the AS group (0.57 episodes per hour or 1 episode every 1.8 hours) was significantly ($p < 0.005$) greater than in the CS group, but not so when compared with the mean frequency in the CONT group ($p > 0.3$). There was no difference in the amplitude of LH pulses between the groups.

FSH levels also fluctuated in an episodic pattern but much less markedly in all groups, so that no significant differences between groups were observed. In general, FSH secretory episodes followed those of LH.

2) GH. As can be seen from Table 4.3 there was no difference in mean GH levels between the groups tested with serial venous sampling. There was also no difference in the pattern of GH secretion over the time of sampling. A substantial proportion of patients from the CS group (75%) and from the CONT group (71%) exhibited no change in GH secretion during the time of sampling greater than the intra assay variation (5%). An episode of GH secretion was seen in 4 CS patients and 5 of the CONT group (in 1 subject from each there was a peak increment > 5 mIU/l). No relationship between these spontaneous increments in GH secretion and gonadotrophin secretion or any other parameter was demonstrated.

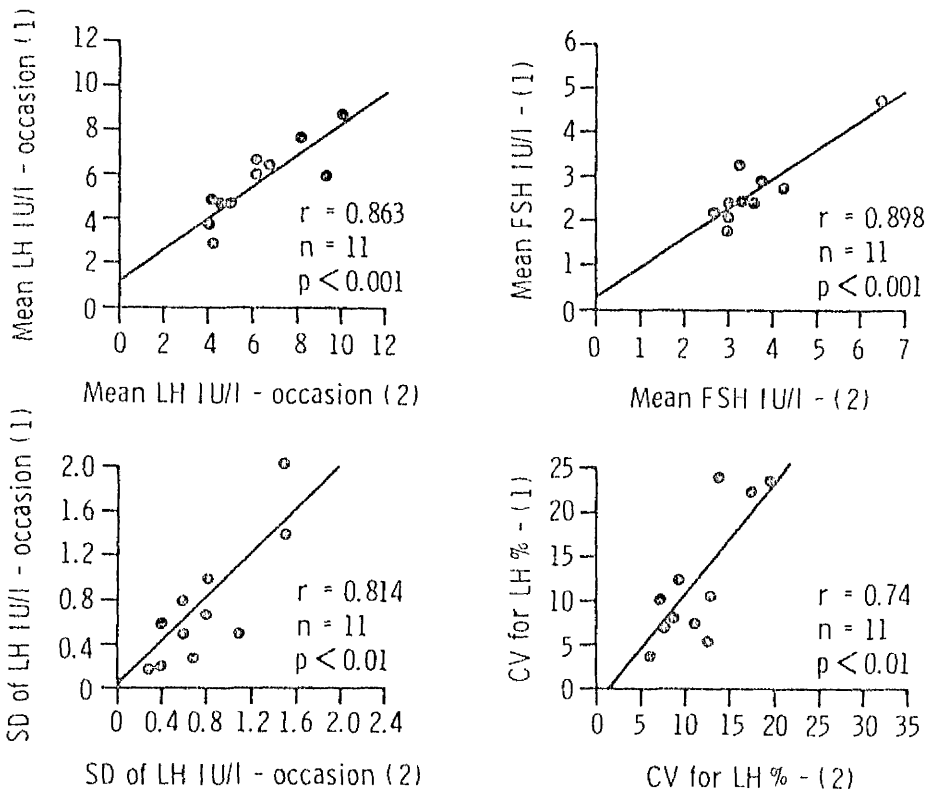
3) PRL. There was a comparable decrement in PRL secretion during the test period (approx. 8 - 11 a.m.) in each of the groups studied (Fig 4.4). There was no statistical difference in PRL level between the groups at any time point. As discussed

Fig. 4.4



Mean serial PRL levels between 0800 and 1100 hrs in 20 chronic schizophrenics and 17 controls. No significant differences between groups.

Fig. 4.5



Relationship between mean LH, FSH, standard deviation (SD) and co-efficient of variation (CV%) of serial LH estimates on two separate occasions in 11 unmedicated chronic schizophrenics.

above a group of CS patients (n = 10) exhibited very little change in LH secretion over the 3 hours of testing (LHCV% of 10% or less: mean 7.3%). It was found that the mean 8 a.m. PRL level in these patients (157 ± 14 (SEM mIU/l) was significantly lower ($p < 0.01$) than those in 10 CS patients whose LHCV% was greater than 10% (mean 24%) (mean 8 a.m. PRL level of this group was 281 ± 39 (SEM) mIU/l).

d) Results of retesting

Eleven patients were retested in a similar way on a subsequent occasion as documented in the methods. The relationship between the mean initial and retest levels of both LH and FSH and the degree of fluctuation of serial LH estimates (measured by calculating the SD and the CV of all estimates)on the two occasions is shown in Fig 4.5. It can be seen that both the levels of LH and FSH and the degree of pulsatility of LH show close intra-individual reproducibility.

3 patients of the original group of 20 CS patients were subsequently started on neuroleptic therapy. These patients were also retested and the results will be described in chapter 6.

e) Correlation between measured variables

1) Hormonal variables. There was a highly significant relationship between LH and FSH in samples from the total group studied ($r = 0.67$, $n = 111$, $p < 0.001$). This relationship was evident in samples from all patient groups. Regression analysis revealed that there was no difference in the slope of this relationship between schizophrenic patients and control subjects.

A positive relationship was established between LH and

testosterone ($r = 0.44$, $n = 30$, $p < 0.05$) in samples from control subjects (CONT group ($n = 17$) and part of the reference population (5a, $n = 13$) but not in samples from any other group.

There was a highly significant relationship between LH CV% and 8 a.m. PRL levels in samples from the CS group ($r = 0.62$, $n = 20$, $p < 0.01$). This relationship also held, though less strikingly, with mean PRL levels ($r = 0.53$, $n = 20$, $p < 0.05$). In other words those patients with little evidence of LH pulsability (low LH CV%) also exhibited lower early morning PRL levels. This relationship was not demonstrated in the CONT group (group 2). No relationship was established between mean LH and FSH levels and 8 a.m. or mean PRL levels in any of the groups studied. A significant inverse relationship between the oestradiol and PRL levels of the same samples was noted in those patients in whom oestradiol measurements were obtained ($r = -0.29$, $n = 70$, $p < 0.05$). This relationship may come about because of the opposite effects of age on these two parameters (see below).

2) Relationship between clinical variables and hormonal data

a) All subjects. Age: There was a significant positive relationship between age and LH and FSH levels in the total population studied (LH: $r = 0.25$, $n = 111$, $p < 0.01$; FSH: $r = 0.23$, $n = 111$, $p < 0.05$). This relationship was also seen in the control group as a whole (groups 2 and 5, $n = 58$; LH: $r = .32$; FSH: $r = .26$, $p < 0.05$) but not in the total schizophrenic group (groups 1, 3 and 4, $n = 53$; LH: $r = .09$; FSH: $r = .18$,

$p > 0.1$) (although a positive trend for increasing gonadotrophin levels with increasing age was noted in the older schizophrenic group). No significant effects of age on LH CV% or on the other hormones were noted. Trends (significant at the $p < 0.1$ level) were noted for oestradiol to increase with age ($r = .29$, $n = 70$) and for PRL to decrease with age ($r = -.21$, $n = 70$). These age effects appear (by calculation of partial co-efficients-see Appendix E) to account for the inverse relationship between PRL and oestradiol mentioned above.

Weight: There was no correlation between the weight of the patients and any of the hormonal variables measured and specifically no weight effect on gonadotrophin secretion or LH pulsatility was shown.

b) Schizophrenic patients. Correlations were examined between clinical variables recorded on the schizophrenic subjecs and measured hormonal data, the more important of which are given below. There are limitations to the relevance of these correlations since a) a large number were examined and therefore a small number may be significant by chance and b) complex interactions may occur between parametric and non-parametric data (for example age and symptomatology) which require more complex statistical evaluation.

1) Length of illness. There was a negative relationship between the degree of LH fluctuation (LH CV%) and length of illness of the CS patients ($r = -0.46$, $n = 20$, $p < 0.05$).

2) Age at first hospitalization. This varied between 16 and 40 years of age in the schizophrenic group under study. There

was a positive relationship between age at first hospitalization and FSH ($r = 0.49$, $n = 53$, $p < 0.01$).

3) Effect of drugs. There was no difference in any of the hormonal variables between the patients who had never received drugs and those who had received minimal or substantial doses in the past.

4) Relationship to mental state. There was a significant, inverse relationship between FSH, but not LH, secretion and positive symptoms in the CS group ($r = -0.47$, $n = 20$, $p < 0.05$ Spearman's rank correlation). No such relationship was noted in the acute schizophrenic group. There was a non-significant trend for decreasing PRL secretion with increasing positive symptoms scores in the acute schizophrenic patients ($r = -0.33$, $n = 30$, $p < 0.1$), but no such trend was seen in the CS patients. There was no significant relationships established between hormonal measures and negative symptom scores, cognitive function scores or Catego classification (see Appendix B) in either group studied.

DISCUSSION

In summary, these results confirm that a number of patients with chronic schizophrenia have low LH and FSH levels compared with an aged matched population. This is associated with a reduction in the frequency, but not amplitude, of LH secretory episodes. Basal levels of PRL, GH, T3, T4, TSH, testosterone and oestradiol were not different between these groups. By contrast, no abnormalities of gonadotrophin or other hormonal secretion

were noted in patients with acute schizophrenia and 3 such patients gave evidence of normal rhythm of LH secretion some time after their acute episode. Some evidence was found to suggest that the abnormal rhythm of gonadotrophin secretion in chronic schizophrenia was associated with low early morning PRL levels and increasing length and severity of illness.

Abnormalities of gonadotrophin secretion cannot be regarded as specific for the diagnosis of schizophrenia. Definite abnormalities of LH and FSH secretion were found only in 50% of the chronic schizophrenics studied and 25% of the patients had both a typical schizophrenic illness and a perfectly normal hormonal profile. Abnormalities of gonadotrophin secretion have been detected in several psychiatric syndromes notably depression (Brambilla et al, 1978), anorexia nervosa (Beumont et al, 1974, b) and neurotic states (Lachelin and Yen, 1978).

The non-specific effects of psychiatric illness which might account for the finding of reduced gonadotrophin secretion in schizophrenia therefore need to be considered first. These include the effects of stress, the effects of prolonged hospitalisation ("institutionalisation"), weight loss and previous drug therapy. The role of these various possibilities will now be discussed in turn.

The patients and controls (some of whom suffered from neurotic illness) were subjected to the same technical procedures of cannulation and repeated sampling. The chronic schizophrenics did not appear "stressed" during the procedure. This however is weak evidence against the notion that stress may be the cause of

the present findings since it has been demonstrated that chronic schizophrenics as a group have evidence of over-arousal on skin galvanic responses while appearing emotionally flat (Deakin et al, 1979). However effects of "stress" on gonadotrophin secretion have not been reported in man though Rose et al (1969) reported reduced testosterone secretion in army personnel under stressful circumstances.

The effects of long term care are a perennial problem in research into chronic schizophrenia because of the difficulties in the selection of appropriate control group. It is noteworthy however that, in the present study, no abnormalities of gonadotrophin secretion were detected in 3 manic-depressive patients hospitalised in the same environment as the CS patients for comparable periods. There is evidence from animal studies that environmental factors can affect gonadotrophin secretion (e.g. Taché et al, 1978) but no comparable evidence is yet available in man. (See chapter 8, for further discussion).

Neuroleptics have been implicated in causing gonadotrophin and/or gonadal dysfunction either directly (Shader and Di Mascio, 1970) or via hyperprolactinaemia (Glass et al, 1975). The evidence for an effect of neuroleptics on LH and FSH is open to debate (Johnstone and Ferrier, 1980) - particularly in the case of chronic neuroleptic therapy (Beumont et al, 1974a), where it has been shown that prolactin levels tend to return to the normal range (De Rivera et al, 1976; Huws and Groom, 1977). In the present study no difference in LH, LH episodic release, FSH or testosterone was noted between patients who had never received

neuroleptics and those who had received substantial amounts in the past.

Weight loss has been implicated in some of the neuroendocrine abnormalities detected in anorexia nervosa and in depression (see chapter 2.2.7). However in the present study no relationship between body weight and the hormonal abnormalities of chronic schizophrenia was established and abnormalities were found in patients whose weight was in the normal range.

It is also unlikely that other psychotropic medication (e.g. antidepressants, anxiolytics) could cause the present findings. Firstly concurrent medication with these drugs was specifically excluded (see chapter 3). Secondly the effects of these drugs on gonadotrophin secretion are weak and variable (Johnstone and Ferrier, 1980 for review). Finally several of the control group (6 subjects) had also been treated with psychotropic medication in the past. Further discussion on the non-specific effects of psychiatric illness on pituitary hormone secretion together with selection of appropriate controls is given in chapter 8.

Before going on to discuss possible causes of gonadotrophin reduction in chronic schizophrenia more specifically related to disease processes, the time scale of the development of these abnormalities will first be discussed. Because no abnormalities of gonadotrophin secretion are seen in patients either with acute schizophrenia or in remission from it and because it appears that increasing length of illness seems to be associated with reduced LH pulsatility, it is tempting to speculate that these

abnormalities relate to the development of impairments of chronic schizophrenia and perhaps therefore have an organic basis (see chapter 1, section 2.4 for summary of the evidence for an organic basis underlying chronic schizophrenia). However there was no relationship between gonadotrophin secretion and accepted measures of impairment in the chronic schizophrenics (e.g. cognitive function tests, negative symptom score). Indeed a negative correlation between FSH secretion and positive symptoms was established in the chronic schizophrenic group (confirming the similar observations of Shader et al, 1968 and Johnstone et al, 1977).

It appears that the age of onset of schizophrenia has an effect on FSH levels estimated many years later: that is, the earlier the age of onset, the lower the estimated FSH. The relationship between the age of onset of schizophrenia, age at puberty and subsequent gonadotrophin secretion is clearly an important area for investigation, though such studies are problematical since the onset of illness is often hard to define and hospitalization policy is variable. The effect of age of onset on FSH may account for the high frequency of gonadotrophin abnormalities in Brambilla's study, as most patients in that study had an onset of schizophrenia in the early and mid teens (Brambilla et al, 1975). These findings are also of particular interest in relation to some neurochemical studies (Spokes et al, 1980) which suggest a particular pattern of biochemical changes in the brains of early onset schizophrenics.

It is also noteworthy that the association of increasing

age with increasing gonadotrophin secretion found in the total normal control group studied here and reported in other studies (Wide et al, 1973; Baker et al, 1976), was not found across the schizophrenic group as a whole. However a non-significant trend for increasing gonadotrophin levels with increasing age was seen in the CS group of patients and therefore the effect was taken into account in analysis of gonadotrophin secretion differences. The lack of an age effect across the total schizophrenic group suggests some change in testosterone/gonadotrophin feedback in schizophrenia as is further discussed below.

Thus no clear answer to the question of the natural history of these abnormalities can be given at present and the relationship of them to the natural history of schizophrenia is similarly uncertain. Further, larger studies will have to be performed to answer these questions. These matters are further discussed in chapter 8.

It appears, from the evidence of this study, that the site of reduced gonadotrophin secretion in chronic schizophrenia lies at the hypothalamic level. This is because there is good evidence that pulsatile LH secretion (reduced in these patients) is prompted by the pulsatile secretion of LHRH from the hypothalamus (Belchetz et al, 1978).

Several factors control the release of LHRH from the hypothalamus. Among the most prominent of these is feedback from gonadal steroids. However neither oestradiol (which in excess is known to reduce episodic LH secretion (Santen, 1975) nor testosterone was abnormal in the chronic schizophrenic group nor

was either statistically related to flattening of the LH secretory profile. An abnormality of the testosterone/LH feedback relationship in schizophrenia has been postulated by Beumont et al (1974). In this regard it is noteworthy that there was a significant positive relationship between testosterone and LH in controls, but not in the schizophrenics and that an age effect on gonadotrophin secretion (seen in controls and thought to be due to the effects of decreasing testosterone feedback with increasing age) did not occur in the total schizophrenic group. The nature of the disturbance in testosterone/gonadotrophin feedback in schizophrenia is not clear but may repay further study.

Animal studies have indicated that several neurotransmitters have effects on pulsatile secretion of LH (Gallo, 1980), but much less evidence is available in man. There is some evidence that opiate agonists reduce (Grossman et al, 1981) and opiate antagonists increase (Moult et al, 1981) the frequency of LH secretory episodes. Noradrenergic, serotonergic and dopaminergic drugs have little effect on LH secretion in man (Johnstone and Ferrier, 1980). However, recent evidence indicates that dopamine (DA) infusions produce lower LH levels and a reduction in the number of episodes of LH secretion (Le Blanc et al, 1976; Kaptein et al, 1980; Huseman et al, 1980), a similar pattern to that seen with dopamine agonists in experimental animals (Gallo, 1980). Thus it is conceivable that the low flat LH secretion pattern of some chronic schizophrenics may reflect overactivity of DA neurotransmission in the hypothalamus or a change in DA receptors in the pituitary. However, if this were the case,

alterations of PRL secretion would be expected. However there was a marked relationship between PRL levels and the LH CV% in the CS patients (such that patients with low PRL also exhibited low LH CV%): both these phenomena might be caused by excess release of DA from the hypothalamus.

Another possible cause of the impairment of gonadotrophin secretion in the chronic schizophrenics relates to ventricular enlargement, which is known to be present in quite a large number of chronic schizophrenic patients and to extend to the third ventricle (see chapter 1, Table 1.2 for review). It is conceivable that hypothalamic damage could result from this enlargement leading to a disruption of releasing factor secretion. However the hormonal deficit found was selective for the gonadotrophins and did not extend to other pituitary hormones e.g. TSH, GH.

Abnormalities of gonadotrophin secretion in chronic schizophrenia have now been reported by several groups of workers. The evidence points to an abnormality in the hypothalamus, either in the LHRH neuronal pathway or some closely related pathway. This ties up with the clinical evidence of hypogonadism in subgroups of chronic schizophrenics cited in chapter 1 (section 2.5). The pathophysiological significance of these findings will be discussed further in the final chapter (8) where the results from all the studies undertaken will be synthesised.

PRL levels were not statistically significantly reduced in the chronic schizophrenic patients studied here which confirms previous observations (Brambilla et al, 1976; Johnstone et al, 1977). In the present study it was also found that the rhythm

of PRL secretion in the early morning was not different from that of controls. It should be noted however that the present study was not principally designed to examine the diurnal rhythm of PRL. To do this comprehensively would require venous sampling while the subject was asleep and recording the exact time of wakening. Times of waking and of getting up were not controlled in the present study. With these reservations in mind, it does appear that PRL is elevated in the early morning and falls to baseline to the same extent in schizophrenics as controls.

It is however interesting that those patients with the lower early morning PRL levels were also those patients who exhibited little evidence of LH episodic secretion (LH CV% of 10% or less - see results). Since this latter phenomon is pathological, this close relationship implies that PRL secretion is also pathologically low in the early morning in these patients (and indeed compared to the controls and remainder of the CS group PRL levels were significantly reduced in these patients).

High levels of early morning PRL levels are thought by some workers (on somewhat limited evidence) to represent a reduction in hypothalamic dopaminergic tone. If this is so, then the low early morning PRL levels seen in some of the chronic schizophrenic patients may represent an overactivity of hypothalamic DA. As was discussed above, there is theoretical evidence to suggest that the excessive DA release or effect could be responsible for the flattening of the LH profile. Other factors may be responsible, but the closeness of the relationship between LH CV%

and 8 a.m. PRL, and the reduction of both these values, in some chronic schizophrenics considered along with the putative role of excess DA in both processes may indicate that the DA over-activity hypothesis of schizophrenia has been confirmed in living patients. However the outstanding problem is that these abnormalities are detected only in a subgroup of patients. Theoretical implications of this are further discussed in chapter 8.

The presence of the trend for lower levels of PRL with increasing age (which has been found by some but not all previous workers) is important since it accounts for the observation that PRL levels are lower in chronic schizophrenic patients compared to acute schizophrenics. This difference was also found in a smaller sample of patients reported in chapter 7 where this observation was of theoretical importance.

No abnormality of basal or episodic GH secretion was detected in the schizophrenic patients. This is in agreement with previously published studies (Cleghorn et al, 1982). Although there is some evidence for dopaminergic influences on GH secretion in man there are significant differences in the pharmacology of GH responses compared to the pharmacology of LH and PRL responses (see chapter 2 section 1.3 and chapter 7).

No abnormalities of thyroid function were detected in the schizophrenic subjects in the present study which confirms previously published studies (Kline et al, 1968; Prange et al, 1979). As was discussed in chapter 1 (section 2.5) abnormalities of thyroid function and schizophreniform illnesses are occasionally related but there seems to be no association between 'typical'

schizophrenia and disturbances of thyroid function.

No abnormalities of hormonal secretion were noted in patients with acute schizophrenia or in acute schizophrenic patients in remission. This is further support for the contention outlined in chapter 1 (section 2.5) that hormonal dysfunction is not an aetiological factor in schizophrenia. FSH was noted to be lower in acute schizophrenics and patients in remission compared with aged-matched controls, but this difference was not significant. The reduction was greatest in those patients with an earlier age of onset. These observations require replication and extension before their significance can be judged.

There was a trend for PRL levels to be inversely related to the positive symptom scores of the acute schizophrenic patients. A similar, but significant, relationship has been noted in a group of chronic schizophrenic patients (Johnstone et al, 1977) and in a group of schizophrenic patients without evidence of ventricular enlargement (Kleinman et al, 1982). This relationship is of great theoretical interest to research workers in biological psychiatry since dopaminergic influences are known to be important in PRL control and are thought to be involved in the genesis of positive symptoms (Crow, 1980).

In summary abnormalities of gonadotrophin secretion have been detected in chronic but not acute schizophrenic patients. These abnormlaities do not appear to be caused by the non-specific sequelae of psychiatric illness such as weight loss, "stress" or "institutionalization". There are several lines of

evidence which implicate overactivity of hypothalamic DA as the cause of these disturbances. Further investigation of these abnormalities are described in Chapters 5 and 6.

CHAPTER 5

Studies on the hormonal response of chronic schizophrenic patients to synthetic hypothalamic releasing hormones

INTRODUCTION

This chapter reports on the pituitary hormonal response to synthetic releasing hormones and refers only to patients with chronic schizophrenia. The main purpose of these studies is to examine whether the abnormalities of gonadotrophin secretion reported here (chapter 4) and elsewhere (Brambilla et al, 1975; Johnstone et al, 1977) have their basis at the pituitary or at the supra-pituitary level.

Brambilla et al (1976) reported reduced FSH and LH in chronic schizophrenia and as part of their investigation of this phenomenon administered LHRH to the patients under study. They reported enhanced LH and FSH responses to LHRH - both in terms of the magnitude and the duration of the response. The patients studied were somewhat unrepresentative of the chronic schizophrenic population in that they all had hebephrenic schizophrenia of very early age of onset (13 - 17 yrs, Brambilla - personal communication). All had recently been withdrawn from neuroleptics. In view of these reservations it was felt that it was necessary to repeat this study in a group of patients more representative of the total chronic schizophrenic population and who had been drug free for much longer periods of time.

The PRL and TSH response to TRH have also been investigated in patients with chronic schizophrenia. There is controversy concerning the magnitude of the PRL response to TRH in schizophrenia

Brambilla et al (1976) finding an enhanced and Prange et al (1979) a normal response. Normal TSH response to TRH has been reported in schizophrenia (Prange et al, 1979) in distinction to patients with affective illness (Asnis et al, 1981).

Abnormal growth hormone secretion following LHRH and TRH administration has been reported in depression (Maeda et al, 1975), anorexia nervosa (Maeda et al, 1976) and in a variety of other conditions, e.g. renal and hepatic failure. A similar abnormal response has been noted in adolescent schizophrenic boys (Gil-Ad et al, 1981) but not in a group of adult schizophrenics (Prange et al, 1977). The hormonal responses to synthetic releasing hormones have been investigated in a group of long stay male schizophrenics all of whom had been unmedicated with neuroleptic drugs for at least a year. Neuroleptic drugs have been shown to alter the PRL response to TRH (Lankford et al, 1981). A full assessment of hormonal status, including serial measurements of LH and FSH, was made to allow comparisons with other groups e.g. men with hypogonadotrophic hypogonadism. The TSH and PRL responses to TRH have been shown to be related to basal steroid levels (Spitz et al, 1979) and these relationships were investigated in the schizophrenic patients.

MATERIALS AND METHODS

Patients studied:

a) Chronic schizophrenic group. 18 of the 20 patients studied in group 1 of the previous chapter were included in this part of the study and were administered LHRH and TRH after the

3 hour period of serial sampling. 2 patients were omitted due to external factors unrelated to clinical state. These patients were therefore selected on the criteria set out in that study namely a) they conformed to the criteria of Feighner et al (1972) for the diagnosis of schizophrenia b) they had not received neuroleptic medication for at least a year prior to study (see Table 5.1 for details) and c) they were agreeable to being studied. Clinical data is similar but not identical with that from the group 1 (chapter 4) and is shown in Table 5.1.

b) Control subjects. 9 of the 17 men in the control group of study 1 were administered LHRH and TRH at the end of the period of serial sampling. They comprised - 3 normal volunteers, 5 patients attending outpatients with a diagnosis of neurosis or reactive depression (all fell into the category of "undiagnosed psychiatric illness" on the Feighner criteria and were symptom free and off medication when studied) and 1 long stay patient of the same hospital as the schizophrenic patients with a diagnosis of manic depressive psychosis (but whose mood was normal and who was off medication at the time of study). The reasons for studying these particular 9 patients out of the group of 17 were random and fortuitous. Further clinical details are given in Table 5.1.

Methods

After an overnight fast a butterfly cannula was inserted in a forearm vein at approx. 8 a.m. and kept open with dilute heparinised saline which was discarded prior to sampling. Samples

TABLE 5.1

Group	n	Weight (kg)	a) Age (yrs)	b) Age at onset of illness (yrs)	Length of illness a - b	Medication
Chronic Schizophrenia	18	66.0 ± 15.3	57.8 ± 7.8 (39 - 68)	25.4 ± 4.8 (18 - 39)	32.0 ± 8.2	Off neuroleptics for 1-12 years (mean 4.5 yrs) 1 on Ibuprofen 1 on Diuretics
Controls	9	75.6 ± 8.1	57.2 ± 9.3 (40- 69)	-	-	1 on Phenobarbitone 50 mg tid Off antidepressant and/or anxiolytic medication for 1/12 - 5 yrs

Clinical characteristics of groups studied.

were taken at 15 - 20 minute intervals until 11 a.m. (a light breakfast was given at 9 a.m.). At 11 a.m. (± 15 min) anintravenous bolus of 100 μg LHRH (Ayerst) and 200 μg TRH (Roche) was administered over 30 seconds and samples taken at +20, +40, +60 and +90 minutes. Side effects were infrequently reported by both groups and included minimal drowsiness and weakness and a desire to micturate. Samples were stored at 4 ^{o}C for 24 hours prior to the separation of sera which was snap frozen over liquid nitrogen and stored at -40 ^{o}C until assay.

Hormone assays

LH, FSH, PRL, GH and TSH were measured by the methods outlined in chapter 3 and in the same series of assays as the studies reported in the preceeding chapter (the same quality control data therefore apply). Testosterone and oestradiol were estimated in the 9 a.m. sample by methods outlined in chapter 3.

Statistics

Serial estimates of LH were made as described above and a mean, standard deviation and co-efficient of variation of LH (LHCV%) were computed as outlined in chapter 4. The area under the response curve for each hormone after LHRH/TRH was computed by subtracting the baseline level (mean of previous 3 readings) from each subsequent level, linear extrapolation and resolution into triangles and rectangles.

Log transformation of the data was carried out when this was required to render the distribution normal. Student's t-test was carried on this data. Correlations were performed by the method of least squares and were performed on untransformed data.

RESULTS

A. Baseline values. Table 5.2 presents the mean level of each hormone estimated for the two groups studied. Results for LH, FSH, PRL and GH are means of serial samples prior to the administration of LHRH and TRH in the schizophrenic and control groups but are in single samples for the remaining hormones.

LH and FSH were significantly reduced in the schizophrenic group ($p < 0.05$ and $p < 0.01$ respectively) with respect to controls but no other differences were noted. Thyroid function (T_3, T_4, TSH) was normal in all subjects.

B. Co-efficient of variation for LH (LHCV%). LHCV% was computed as outlined above. The mean levels for the two groups is shown in Table 5.2. There was a significant difference ($p < 0.05$) between the two groups (i.e. the samples from controls showed greater fluctuation of LH than those from the schizophrenic patients).

C. Response to TRH/LHRH.

1) LH, FSH, TSH, PRL. These results are presented in Fig 5.1 which depicts the pituitary responses in the two groups according to time and in tabulated form in Table 5.3.

It can be seen that the FSH response to LHRH is reduced in the schizophrenic group both in terms of the area under the curve ($p < 0.01$) and in terms of maximal increment ($p < 0.05$). The PRL response to TRH is similarly blunted ($p < 0.05$ and $p < 0.05$ respectively). The LH response to LHRH and the TSH response to TRH were non-significantly reduced in the schizophrenic group.

TABLE 5.2

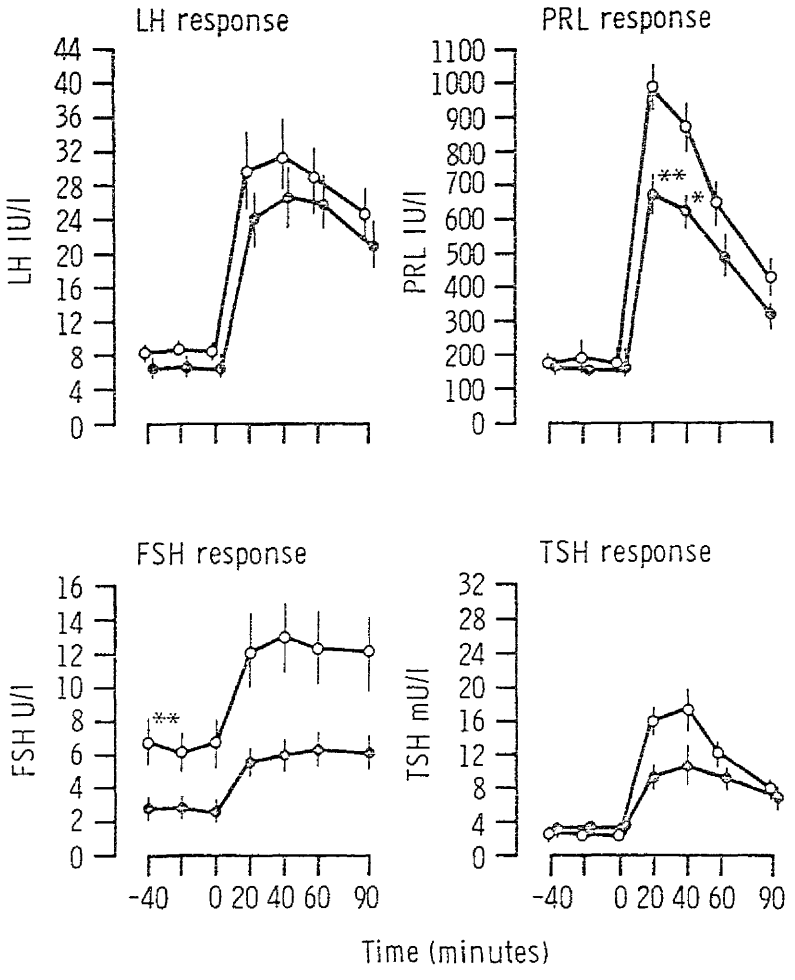
Group	n	GH mU/l	FSH U/l	LH IU/l	PRL mIU/l	T_3 n mol/l	T_4 n mol/l	TSH mU/l	Testosterone n mol/l	E_2 n mol/l	LHCV %
Chronic Schizophrenia	18	1.5 ±0.4	2.8 ±0.4	6.1 ±0.7	154.4 ±20.4	2.1 ±0.1	78.6 ±3.0	3.5 ±0.5	25.6 ±2.2	72.6 ±6.1	12.1 ±2.8
Controls	9	1.1 ±0.2	6.9** ±1.5	8.7* ±1.1	174.2 ±36.3	-	-	2.9 ±0.3	26.5 ±2.9	66.6 ±10.7	19.7* ±2.5

Mean levels of hormones in 18 chronic schizophrenics and 9 controls in period prior to LHRH/TRH administration. * $p < 0.05$, ** $p < 0.01$ compared to chronic schizophrenics.

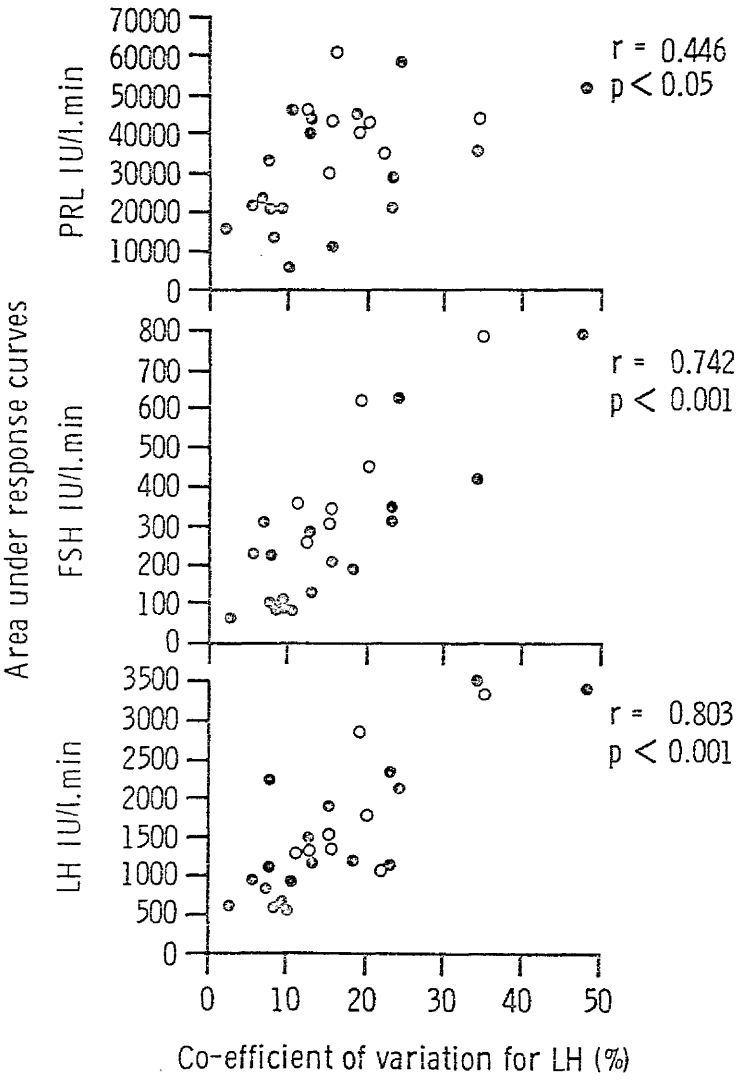
TABLE 5.3

HORMONE	LH			FSH			PRL			TSH		
	Baseline IU/l	Peak Δ IU/l	Area IU/l. min	Baseline U/l	Peak Δ U/l	Area U/l. min	Baseline mIU/l	Peak Δ mIU/l	Area mIU/l. min	Baseline mU/l	Peak Δ mU/l	Area mU/l. min
Chronic Schizophrenia n = 18	6.3 ±0.8	22.4 ±3.0	1503.2 ±212.1	2.7 ±0.3	4.2 ±0.7	256.1 ±45.0	159.8 ±20.1	546.4 ±55.6	30374 ±3510	3.5 ±0.5	9.5 ±2.2	537.5 ±128.4
Controls n = 9	8.6 ±1.0	23.5 ±4.2	1605.2 ±295.1	7.0 ±1.4	6.8 ±1.1	434.1 ±83.1	190.1 ±38.4	806.4 ±62.9	42990 ±3375	2.9 ±0.3	15.8 ±3.0	857.4 ±136.9

Basal, peak increment (Δ) and area under response curve of LH, FSH, PRL and TSH following LHRH/TRH administration in 18 chronic schizophrenics and 9 controls. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to chronic schizophrenics (basal level are mean of 3 levels prior to LHRH/TRH).



Mean LH, FSH, PRL and TSH secretion following LHRH/TRH in 18 chronic schizophrenics (closed circles) and 9 controls (open circles). FSH reduced at time points and PRL at +20 and +40 minutes. ** $p < 0.01$ * $p < 0.05$ (SE)



Relationship between area under the PRL, FSH and LH response curves and fluctuation of LH in serial samples in 18 chronic schizophrenics (closed circles and 9 controls (open circles)

2) GH. GH results are shown in Tables 5.4 and 5.5. It was noted that there was a GH increment following LHRH/TRH administration in 9 out of the 18 schizophrenic patients, but none of the control subjects. Comparison was made by calculating the percentage change from baseline (mean of three levels prior to LHRH/TRH administration) of each subsequent GH estimation in each subject. 9 of the schizophrenics had percentage increments that were greater than the control mean plus 2 SD of the mean (95% confidence limit). The timing of this increment was variable. In 5 of the patients it was evident at either +20 or +40 minutes post LHRH/TRH or both. In the remainder it was noted at either +60 or +90 minutes. Due to this variability of response, no significant difference in GH level between schizophrenics and control were found at any time point during the test procedure.

No evidence of spontaneous fluctuation of GH prior to LHRH/TRH administration was noted in either group and no subjects had high baseline GH levels.

4 of the schizophrenic patients had a very marked increment of GH secretion (> 300%) of baseline. It was found that these patients had significant reductions in the size of the LH response to LHRH ($p < 0.01$) and PRL response to TRH ($p < 0.01$) compared with the remainder of the schizophrenic subjects who exhibited no or slight rise in GH secretion following LHRH/TRH administration (Table 5.5). The size of the FSH increment following LHRH, and the basal LH, FSH, PRL and LHCV% levels, were also reduced, but not significantly in GH responders. There was no significant relationship between change in GH secretion and LH, FSH or PRL

TABLE 5.4

Group		Time after LHRH/TRH administration (mins)				
		0	20	40	60	90
Chronic Schizophrenia n = 18	GH mU/l	1.5 ±0.4	2.5 ±0.8	2.1 ±0.7	1.5 ±0.4	1.7 ±0.4
	GH as Percentage of time 0	100	174 ±48.5	170 ±48.7	119 ±27.1	145 ±36.0
Controls n = 9	GH mU/l	1.1 ±0.2	1.0 ±0.2	1.0 ±0.1	1.0 ±0.2	1.0 ±0.1
	GH as percentage of time 0	100	97 ±3.6	99 ±7.9	95 ±4.1	95 ±5.0

GH in mU/L and as percentage of time 0 in 18 chronic schizophrenics and 9 controls at various intervals after LHRH/TRH administration.

TABLE 5.5

Group	FSH U/l	FSH Response Area U/l. min	LH IU/l	LHCV %	LH Response Area IU/l. min	PRL mIU/l	PRL Response Area mIU/l. min
Schizophrenic patients (n=4) with large increment in GH (>300% baseline) after LHRH/TRH administration	2.5 ±0.4	174 ±57	4.8 ±0.7	10.6 ±4.6	831** ±132	168 ±37	16269** ±3505
Schizophrenic patients without large movement in GH secretion (n=14)	2.9 ±0.4	280 ±57	6.5 ±0.8	17.2 ±3.2	1695 ±254	217 ±28	34392 ±3933

Comparison of basal and stimulated gonadotrophin and PRL secretion between chronic schizophrenics with and without a large increment in GH secretion post LHRH/TRH. ** $p < 0.01$.

secretion following LHRH/TRH administration across the whole group of schizophrenics.

D. Correlation between hormonal variables. Significant correlations are set out in Table 5.6 in the form of a correlation matrix.

Basal LH, FSH: There was a significant relationship between basal LH and basal FSH and between each of these hormones and the area of LH response and the FSH response to LHRH.

Basal PRL: There was a highly significant relationship between basal PRL and the area of the PRL response curve after TRH.

Testosterone/Oestradiol: Neither testosterone nor oestradiol related to any other hormonal variable or response but there was a significant positive relationship between them.

Co-efficient of variation for LH (LHCV%): There was a highly significant relationship between the degree of fluctuation of LH estimates prior to releasing hormone administration and the size of the area under the LH, FSH and PRL response curve (Fig 5.2). As can be seen from fig 5.2 these relationships between LHCV% and size of the LH and FSH area under the curve held for both schizophrenic and control subjects (LH: $r = 0.82$, $n = 18$ chronic schizophrenics, $p < 0.001$: $r = 0.72$, $n = 9$ controls, $p < 0.05$; FSH: $r = 0.84$, $n = 18$, $p < 0.001$: $r = 0.91$, $n = 9$, $p < 0.001$). However the relationship between LHCV% and PRL response area was non-significant in the case of the control subjects ($r = 0.46$, $n = 9$, $p > 0.1$) whereas it was highly so in the case of the schizophrenic patients ($r = 0.64$, $n = 18$, $p < 0.01$).

FIG 5.3

	Age	Weight	LH	FSH	PRL	TSH	Testosterone (T)	Oestradiol (E2)	LHCV %	LH area (▲)	FSH area (▲)	PRL area (▲)	TSH area (▲)
Age													
Weight													
LH													
FSH			.69										
PRL	-.38												
TSH													
Testosterone (T)		.											
Oestradiol (E2)							46						
LHCV %	-.44		.52	.38									
LH area (▲)			.60	.48					.80				
FSH area (▲)			.54	.58					.74	.79			
PRL area (▲)					.50				.45				
TSH area (▲)													

r .38 - .49 = p 0.05-0.01 n = 27
r .49 - .60 = p 0.01-0.001
r > .6 = p < 0.001

Correlation matrix demonstrating relationships between basal and stimulated hormone levels before and after LHRH/TRH administration in total group studied. (n = 27). Area (▲) refers to area under response curve.

It was also noted that there was a significant relationship between LHCV% and basal LH, FSH but not PRL levels i.e. patients with reduced basal gonadotrophin levels also tended to have reduced spontaneous LH fluctuation.

In addition there was a highly significant relationship between the LH, FSH and PRL responses i.e. the higher the response in one hormonal variable the higher the other hormonal responses. There was no statistical relationship between any of the hormonal measurements and patient age or weight (note - the ranges of age and weight of the groups studied were rather narrow).

Patients only: There was no relationship between any of the hormonal variables post LHRH/TRH and clinical characteristics of the patients such as length of illness, age at onset of illness and positive and negative symptoms (ratings made as described in chapter 3).

DISCUSSION

The results of this study indicate that in chronic schizophrenia, in addition to reductions in LH and FSH secretion and reduced spontaneous fluctuations of LH, the hormonal response to synthetic releasing factors was disturbed with a reduced FSH response to LHRH, a reduced PRL response to TRH and GH increments after LHRH/TRH in a number of patients. These changes were highly inter-related: patients who had one abnormality were likely to have the others.

There was considerable variability in hormonal results in both groups. Abnormalities were observed only in a subgroup of

the schizophrenics (approx. 50%) and 25% of the schizophrenic patients were in the normal range on every variable measured. The cause of this hetereogenity is not clear at present. Mis-diagnosis appears unlikely as all the patients studied appeared to have had classical schizophrenic illnesses. A similar situation is encountered in studies on patients with affective illness (see chapter 2.2.2). The problems of interpretation raised by this heterogenity of hormonal response are further discussed in chapter 8.

It should be emphasized that these findings refer only to a group of patients hospitalised for many years. The effects of long term care and non-specific effects of psychiatric illness are a perennial problem in research into chronic schizophrenia because of the difficulties of selecting an appropriate control group. Theoretical spects of this problem and practical attempts carried out to circumvent these problems are more fully discussed in the concluding chapter (8).

Spontaneous episodic secretion of LH and to a lesser extent FSH is a well recognized phenomenon. Serial venous sampling techniques have revealed that there are 12 - 20 bursts of LH secretion per day in men (Santen and Bardin, 1973). There is good evidence that this pattern of gonadotrophin secretion from the pituitary is regulated by pulsatile secretion of LHRH from the hypothalamic median eminence (Belchetz et al, 1978). Serial LH estimates revealed that the frequency of spontaneous episodes of LH secretion was reduced in the schizophrenic group as a whole and was almost completely absent in a subgroup of patients.

There was a significant relationship between LHCV% (a measure of the degree of fluctuation of serial LH estimates) and the size of the FSH and LH response to synthetic releasing hormones in the total group studied and both groups separately. A similar relationship between LHCV% and the size of the PRL response in the total group and the schizophrenic group and a similar tendency in the controls was also demonstrated. This indicates that those patients with reduced stimulated hormone responses were also those who exhibited little spontaneous fluctuations of LH. Since this latter phenomenon is most likely to be secondary to reduced spontaneous secretion of LHRH from the hypothalamus, these close relationships may indicate that the reduced hormonal response to synthetic releasing hormones in schizophrenia may have their basis in reduced spontaneous endogenous releasing hormone secretion. However, while this is a conceivable pathophysiological explanation for the reduced FSH response to LHRH, it does not explain why the LH response was not significantly reduced from control levels, nor does it explain the reduced PRL response to LHRH/TRH administration.

Reduced spontaneous secretion of hypothalamic LHRH is though to be the underlying pathophysiological process in hypogonadotrophic hypogonadism (Spitz et al, 1979). It would be of considerable interest to investigate whether the repeated administration of exogenous LHRH to schizophrenic patients restores normal FSH responsiveness to LHRH as has been shown to be the case in hypogonadotrophic hypogonadism (Jacobsen et al, 1980) and anorexia nervosa (Marshall and Kelch, 1979).

There are however, several important distinctions between the hormonal results of the schizophrenic patients and patients with hypogonadotrophic hypogonadism (also called isolated gonadotrophin deficiency or IGD). IGD patients also have a reduced PRL response to TRH, but this has been shown to be a consequence of the altered steroid milieu found in these patients (reduced oestradiol and testosterone levels) as treatment of these patients with hCG or testosterone restores PRL responsiveness to TRH to normal (Spitz et al, 1979). By contrast testosterone and oestradiol were not abnormal in the schizophrenic patients and blunted PRL responses were found to relate to the reduced spontaneous fluctuation of LH and not to testosterone or oestradiol levels. Similarly IGD patients exhibit a markedly reduced LH but normal or exaggerated FSH response to LHRH (Roth et al, 1972) whereas the schizophrenic patients have a normal LH but reduced FSH response.

The endogenous release of hypothalamic releasing factors is under complex control and pituitary responsiveness to these agents is a complex and incompletely understood phenomenon. Monoamine neurones innervate the hypothalamus and are known to be related to hypophysiotropic neurones, anatomically and functionally (see chapter 2). Some of the changes noted here could conceivably be related to an excess release of DA from the hypothalamus e.g. DA infusion has been reported to lower LH levels and reduce the frequency of LH secretory episodes (Huseman et al, 1980) and DA infusions are known to reduce the PRL response to TRH (Besses et al, 1975). Brambilla et al (1975) reported a

return of LH and FSH levels to the normal range in schizophrenic patients after the administration of DA blocking therapy. In support of the notion that overactivity of DA could be responsible for reduced LH secretion in some circumstances it has recently been observed that acute DA blockade with metaclopramide restores LH secretion to a more normal pattern in female patients with hypothalamic amenorrhoea (Quigley et al, 1980).

However there are several lines of argument against the hypotheses that overactivity of DA could be responsible for the present findings. For example, the normality of basal PRL levels is strong evidence against this hypothesis. Furthermore DA infusions are known to reduce the TSH response to TRH (Besses et al, 1975), but this response was normal in this study.

The pathophysiological significance of abnormal GH hormone secretion following administration of LHRH and TRH in a variety of apparently disparate syndromes is uncertain. Animal evidence indicates that this response occurs when there is "hypothalamic-pituitary disconnection " (Muller et al, 1979).

In this regard, it is noteworthy that those schizophrenic patients with marked GH secretion after LHRH/TRH administration also exhibited markedly reduced LH and PRL responses (Table 5.5). In other words these patients had the most evidence of hypothalamo-pituitary derangement.

Gil-Ad et al (1981) described marked GH elevations in a group of adolescent schizophrenic boys (mean age 15 y 8 months) and found that neuroleptic drugs reversed the GH response to LHRH but not to TRH. Since Prange et al (1979) did not find GH

secretion after TRH in their group of adult schizophrenic patients this raises the possibility that the early age of onset of Gil-Ad's patients is the critical factor. However there was no difference in the present study between the age of onset of schizophrenia of those patients who exhibited abnormal GH increments and those who did not. The determination of the age of onset of schizophrenia is, however, often a difficult clinical problem especially in groups of patients where the duration of illness is long.

CAT scan evidence of ventricular dilatation has been found in cases of chronic schizophrenia (see Table 1.2). There is evidence from echo-encephalographic, pneumoencephalographic and CAT scan studies that dilatation of the 3rd ventricle may occur in chronic schizophrenia (see Table 1.3, chapter 1). It is conceivable that damage to the hypothalamus could result from this enlargement leading to derangement of releasing factor secretion. However if hypothalamic damage was the cause of the results reported here then disruption of all aspects of pituitary secretion (e.g. basal TSH and PRL secretion) would be expected but does not occur.

The results reported here differ from those reported by Brambilla et al (1976) (who found exaggerated LH and FSH responses to LHRH) and to those of Prange et al (1979) (who found a normal PRL response to TRH in schizophrenia). Possible reasons for the discrepancies are that both the above studies were performed on patients recently withdrawn from drugs (in distinction to the minimum of a year and mean of 4.5 yrs (Table 5.1) off medication

in the present study) and the different dose regimens of releasing factors employed. For example the dose of TRH employed here was 200 µg which is below that required to produce a maximal TSH response (400 µg) and below used by Prange et al (1979).

LHRH and TRH were administered concominantly in the present study (as is the practise in conventional endocrine diagnostic studies). However in research studies in which abnormal responses to releasing hormones are seen (e.g. in depression (Brambilla et al, 1978)) it is preferable to administer LHRH and TRH separately and studies with schizophrenic patients need to be repeated in this way.

In summary, the administration of LHRH and TRH to chronic schizophrenics revealed some abnormalities of hormonal response which have similarities with, but some important differences from, other clinical and diagnostic groups. The pattern of the abnormalities noted and their clear relationship to abnormalities of pulsatile LH secretion indicates that the level of disruption is above the pituitary level and most probably resides in the hypothalamus. This study indicates ways in which the neurohumeral disturbance of these particular patients could be further investigated and perhaps elucidated.

CHAPTER 6

Effect of DA blocking drugs on anterior pituitary hormone secretion in acute and chronic schizophrenia

INTRODUCTION

Neuroleptic drugs are effective in relieving schizophrenic symptoms. This has been shown to be specifically related to the DA blocking properties of these drugs and to be unrelated to their other pharmacological effects e.g. their anticholinergic or sedative poperties (see chapter 1.2.3 for references). There has been much recent interest in the study of anterior pituitary hormone secretion (APHS) in response to neuroleptic drugs in schizophrenia for the following reasons: 1) a change in hormone secretion may act as an index or marker of drug level and/or therepeutic efficacy and 2) to investigate whether abnormalities of APHS in schizophrenia are DA-related.

PRL secretion has been extensively studied in schizophrenia because of the importance of the TIDA neurones in inhibiting PRL secretion (Meites et al, 1972). It is generally accepted that PRL secretion is normal in acute (Meltzer et al, 1974; Ettigi et al, 1976) and chronic (Brambilla et al, 1976; Johnstone et al, 1977) schizophrenia and there is evidence that the PRL response to acute neuroleptic administration in patients with schizophrenia is similar to that found in controls (Gruen et al, 1978). Whether the PRL response to chronic neuroleptic therapy and whether tolerance to PRL raising effect of neuroleptics varies between schizophrenics and controls has not been tested.

Several research groups have investigated the clinical correlates of the PRL response to neuroleptics. Rao et al (1980) found significant relationships between the dose of neuroleptic prescribed, plasma level of neuroleptic and PRL level in a series of female chronic schizophrenics on stable doses of haloperidol. However in two series of schizophrenic patients treated on an acute basis with chlorpromazine (Kolakowska et al, 1975; Kolakowska et al, 1979) a significant relationship between plasma neuroleptic level and PRL was found, but relationships with dosage were not established. In these latter studies the numbers were relatively small and the dosage of chlorpromazine was determined by clinical judgement.

A positive relationship between the antipsychotic effects and PRL elevating effects of neuroleptics has been established (Meltzer and Fang, 1976; Langer et al, 1977; Cotes et al, 1978). However this association is not strong: some patients show a good response with little change in PRL secretion and vice versa. Moreover because there is a ceiling effect, this relationship may not be apparent at all doses (Gruen et al, 1978).

The relationship between PRL levels and extrapyramidal side effects of neuroleptics has been less widely studied. Rao et al (1980) did not find the two significantly related. Wiles et al (1976) found a non-significant tendency for extrapyramidal side effects to be associated with higher PRL levels and a similar tendency significant in some but not all comparisons was found in the study of Kolakowska et al (1979). It has been shown that PRL rises (Glazer et al, 1981) and falls (Brown et

al, 1979) to a greater extent and more rapidly in those patients with neuroleptic induced neurological side effects. The problems of studying this type of relationship is that the patient populations tend to be heterogenous and include patients of variable age, sex and past neuroleptic experience and it may be that these factors have tended to obscure or create relationships between extrapyramidal effects and PRL which might not otherwise be found.

Another problem in correlating PRL secretion, neuroleptic blood levels and clinical effects is the differential effects on these variables of other drugs prescribed during the treatment of acute schizophrenia. For example some (Rivera Calimlin et al, 1976) but not all (Simpson et al, 1980) studies have found that anticholinergic medication (prescribed in treatment of or prophylaxis against the neurological side effects of neuroleptics) reduce plasma neuroleptic levels. Lal et al (1979) found that anticholinergic medication enhanced the PRL elevation induced by acute neuroleptics. However de Rivera et al (1976) found that anticholinergics had no effect on PRL levels in patients treated acutely with neuroleptics (although an enhancement was observed during chronic neuroleptic treatment).

The effects of neuroleptic treatment in LH and FSH secretion in schizophrenia have been less widely studied and are the cause of some dispute. A few studies have reported reduced LH and FSH after neuroleptic administration (Shader and de Mascio, 1970) but several others have not (e.g. Beumont et al, 1974a). Animal studies have generated similarly conflicting data

(Weiner and Ganong, 1978). However in the case of LH and FSH secretion in schizophrenics treated with neuroleptics the situation is more clearcut. Several studies have shown no effect of neuroleptic administration on LH and FSH where they have been prescribed for the treatment of acute psychotic episodes (Beumont et al, 1974a; Cotes et al, 1978). Chronic neuroleptic treatment was reported by Brambilla et al (1976) to be associated with an elevation of LH and FSH levels in chronic schizophrenics. On the other hand Beumont et al (1974a) found no change in LH and FSH secretion after chronic neuroleptic withdrawal (but reported that testosterone rose during this period).

An interesting finding has recently been reported by Yen's group of colloborators (Quigley et al, 1980). They found in female patients with stress induced amenorrhoea that the acute administration of a DA blocking drug elevated the low LH but not the low FSH secretion characteristic of this condition. No effect on LH and FSH levels of normal controls was noted. There was also some evidence of a return of episodic LH secretion after DA blockade. This work ties in well with the observations that DA administration reduces LH but not FSH secretion and reduces the frequency of LH secretory episodes in normal controls (Kaptein et al, 1980). This implies that the mechanism of amenorrhoea following stress in the female is dopaminergic over-activity.

Acute DA blockade was achieved in the study of Quigley et al (1980) by the intravenous administration of metoclopramide (MCP). This drug is commonly prescribed as an anti-emetic.

The pharmacological profile of MCP is principally one of blockade of DA receptors. There is an unconfirmed report from an open study (Stanley et al, 1981) that regular oral medication with metoclopromide is therapeutically effective in the treatment of schizophrenia.

This chapter reports on three separate studies which investigate the effects of neuroleptic administration in schizophrenia. The first two studies investigate respectively the effect of chronic (Study 1) and acute (Study 2) DA blockade on APHS in chronic schizophrenia. The third (Study 3) investigates the relationships between neuroleptic blood levels, APHS, the clinical effects of neuroleptics and the effect of additional anticholinergic medication over a wide range of neuroleptic dosage in acute schizophrenics.

MATERIALS AND METHODS

Patients studied

Study (1). 3 patients were studied. These patients were part of the group of 20 chronic schizophrenics described in chapter 4 who had been studied by a 3 hour period of serial venous sampling. Some months after the original study, neuroleptic therapy was prescribed by the medical teams responsible for the care of these patients. These psychiatrists were unaware of the findings of the original study. This group were representative of the main original group in terms of age and length and severity of illness. The clinical details of these patients, together with the doses of neuroleptics administered can be seen in Table 6.1.

These patients were restudied after several months of therapy by exactly the same methods as on the first occasion (see below). At the time of the retesting there was no discernable difference in the clinical state of these patients following the introduction of neuroleptic therapy.

Study (2). Acute DA blockade in male chronic schizophrenics and controls with metaclopramide (MCP). 10 chronic schizophrenics and 10 controls took part in this study. The chronic schizophrenics conformed to the criteria for selection set out in chapter 4 which are briefly that a) they conformed to the criteria of Feighner et al (1972) for the diagnosis of schizophrenia b) none of them had had neuroleptic medication for at least a year prior to study c) they were physically healthy and d) they were agreeable to study. Clinical data are given in Table 6.1.

10 controls were studied. All were men who had previously attended outpatient clinics at Northwick Park Hospital with a diagnosis of neurotic illness, reactive depression or personality disorder, none of these being severe. Examination of their casenotes by the Feighner criteria lead to them being placed in the "unspecified psychiatric disorder" category. Several had received a course of benzodiazepines and/or tricyclic antidepressants but all had been off medication for several years at the time of study (see Table 6.1). One patient was receiving diuretics for the treatment of mild hypertension. The day to day functioning of these patients was unimpaired and most were working at the time of study.

Study (3). Relationship between APHS, neuroleptic level

and clinical features in patients with acute schizophrenia treated with and without anticholinergic medication. 36 patients were studied (22 males and 14 females). All had been admitted to the research ward of the Clinical Research Centre with acute schizophrenic symptoms (developing within the month preceeding admission). Only patients with nuclear schizophrenic symptoms (as defined by the Present State Examination) were included. Details of previous psychotic illnesses, presence of family history and previous drug administration are documented in Table 6.1. 2 of these subjects refused to participate in the study sometime during the first week of study and are excluded from further analysis.

Methods

Drug regimes and sampling schedules

Study (1). 3 patients were studied. They were tested in a similar manner to that described in chapter 4 i.e. an IV cannula was inserted in the fasting state and serial venous samples withdrawn at 20 minute intervals for 1 - 3 hours. Since the patients were fasting they had not ingested their neuroleptic medication for approximately 14 hours prior to the first sample (see Table 6.1 for details). One patient could not be sampled as fully as the first occasion due to external factors.

Study (2). 20 patients were all studied in an identical manner. After a light breakfast at 8 - 8.30 a.m. a butterfly cannula was inserted at 9 a.m. serial samples obtained at 15 minute intervals for 3 hours (13 samples) in the manner described in the methods section in chapter 3. After 90 minutes of

Study	Diagnosis	Age and Sex	Clinical details	Drug History
1	Chronic schizophrenics n = 3	58 ± 1.3 3♂	Chronically hospitalised (mean length of illness 33 yrs)	Patient A Unmedicated till 6/12 ago. → CPZ 50 mg tds Patient B Stopped neuroleptics 3 yrs ago. 3/12 ago → Thiordiazine 25 mg tds Patient C Unmedicated 3/12 → Thiordiazine 50 mg bd
2	Controls n = 10	56 ± 2.7 10♂	Patients with history of neurosis or reactive depression. Symptom-free	2 on Benzodiazepines 1 on Diuretics
	Chronic schizophrenics n = 10	60 ± 2.9 10♂	Chronically hospitalised (mean length of illness 32 yrs)	6 off neuroleptics for 1 - 12 yrs 4 never medicated 1 on Diuretics
3	Acute schizophrenics n = 36	30 ± 1.8 22♂ 14♀	Patients with acute symptoms in past month, 1st episode for 15, >1 episode for 21. +ve FH in 12 patients	26 off medication for >1/12 10 receiving neuroleptics in month prior to study

Clinical details of main groups tested with neuroleptics.
Age as mean ± SEM.

TABLE 6.2

Variable	Means of Assessment	Days of Assessment
Sedative Effect	Nursing ratings of state of wakefulness every ½ hour of 24 hours	daily for 56 days of study
Mental State	Krawiecka Scale (1977) (incongruity & flattening of affect separated to give a total of 9 variables)	0 (i.e. day before commencement of drugs), 7, 10, 14, 17, 21, 28, 28
Extrapyramidal Effects	CRC Extrapyramidal Rating Scale (Johnstone et al, 1970)	0, 4, 7, 10, 14, 17, 21, 28, 28
Plasma Flupenthixol	radioimmunoassay (see below)	0, 2, 10, 14, 21, 28
Serum levels of anterior pituitary hormones	radioimmunoassay (see below)	0, 7, 14, 14, 21, 28

Schedule of assessments carried out in Study 4 (effect of flupenthixol on symptoms, motor behaviour, plasma flupenthixol and anterior pituitary hormone secretion in 36 acute schizophrenics (see above)).

sampling (7 samples) 10 mg of metoclopromide (Primperan : MCP) was injected intravenously over a 2 minute period. There were no marked clinical effects of this injection other than minimal drowsiness in a few of the subjects from both groups.

Study (3). These patients were studied over a four week period. These patients were blindly allocated to one of three groups treated with capsules containing either 1 mg, 2 mg or 4 mg flupenthixol. The number of capsules prescribed was that required to produce 10 hours sleep in 24 hours. The dose was increased by one capsule every 2 days and adjusted to this level over the first 10 days of the study and was thereafter fixed for the remaining 18 days of the study. At 10 days either procyclidine 5 mg tid or identical placebo was randomly and blindly allocated to equal numbers of patients. No additional neuroleptic medication was prescribed throughout the trial but the design of the trial did allow for the administration of intravenous procyclidine (10 mg) if required to manage dystonic neuroleptic side effects. Assessments carried out included clinical ratings, measurements of extrapyramidal side effects, and venous sampling for anterior pituitary hormone secretion and flupenthixol blood levels. The schedule of these assessments is summarised in Table 6.2

Measurements

A) Laboratory

1) Anterior pituitary hormone secretion. LH, FSH and PRL were estimated by the methods outlined in chapter 3. In the case of study 1, LH and FSH were estimated in one assay.

This assay also included samples from the first testing session of these patients and there was a close inter-assay concordance with previous assays. Study 2: LH, FSH and PRL were measured in one of three assays which contained samples from patients and controls and a range of quality controls and duplicate samples with acceptable inter-assay correlation of variation (see chapter 3). Study 3: LH and FSH samples were both estimated in a single assay. PRL was measured in 3 separate assays each of which included samples from several treatment groups. All samples from each patient were measured in one assay. The mean co-efficient of variation between estimations for QC's tested in each of the three assays (n = 5) was 7.8%.

2) Flupenthixol assays. Flupenthixol was measured by Miss R. Bourne, Research Officer, CRC, under the supervision of Dr. P.M. Cotes and myself. Flupenthixol was estimated in heparinised plasma by a modification of the radioimmunoassay method of Robinson and Risby (1977) using an antiserum supplied by Dr. A. Jorgensen. Duplicate samples of plasma (0.1 ml) were incubated for 60 hours at 4 ^{o}C with 1.44 nN (^{3}H) cis (z)-flupenthixol (^{3}H-flupenthxiol) and antiserum (final dilution 1:120,000). Bound and free radioactivity were separated using 0.2 ml dextran coated charcoal (Dextran T-40 0.025% W/V and Norit A charcoal 0.25 W/V) at 4 ^{o}C, followed by centrifugation. The bound fraction, in the supernatant, was measured by liquid scintillation counting. Duplicate tubes for each sample containing no antiserum were also assayed to estimate non-specific binding which was subtracted from the bound sample counts.

Sample concentrations were estimated from a log-logistic plot of a standard curve of α-flupenthixol serially diluted in heparinised drug-free plasma. The assay has a working range of 1 - 50 ng flupenthixol/ml plasma. Flupenthixol was estimated in 5 separate assays, each of which included samples from several treatment groups. All samples from each patient were estimated in a single assay. The mean coefficient of variation for a range of quality controls (made up from a pool of heparinised plasma samples from patients receiving flupenthixol) was 13%.

B) Clinical ratings

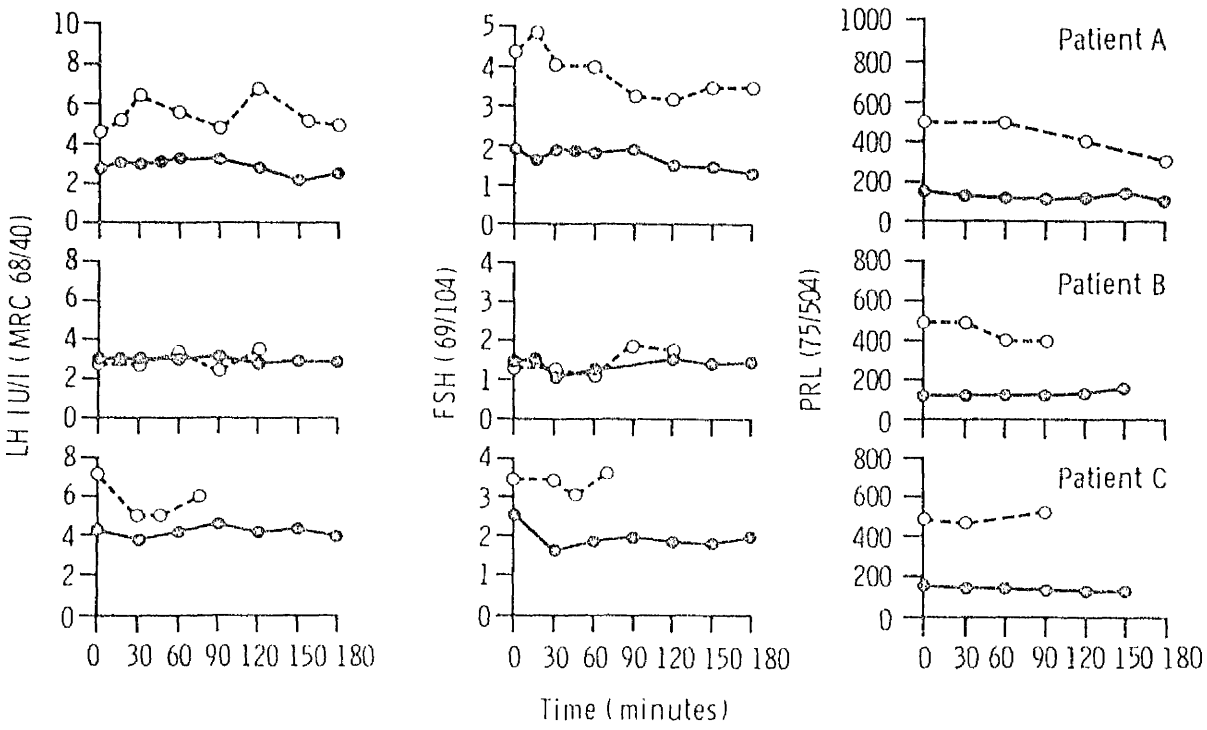
1) Mental state was rated twice weekly, the rating scale devised by Krawiecka et al (1977) as described in chapter 3.

2) Movement and tone were rated with a simple scale (CRC Extrapyramidal Rating Scale - Owens and Johnstone (1980)) rating a) general motor restlessness, b) facial dyskinesia, c) facial rigidity, d) facial tremor, e) upper limb rigidity, f) hand tremor, g) abnormality of gait, each on a four point scale.

RESULTS

Study (1). The results of this study are depicted in Fig 6.1 which shows the results of serial LH, FSH and PRL from these particular patients on the two occasions studied. It can be seen that in all the patients there was a slight rise in PRL secretion on the second occasion of testing following the prescription of neuroleptics. It was not possible, due to

Fig. 6.1



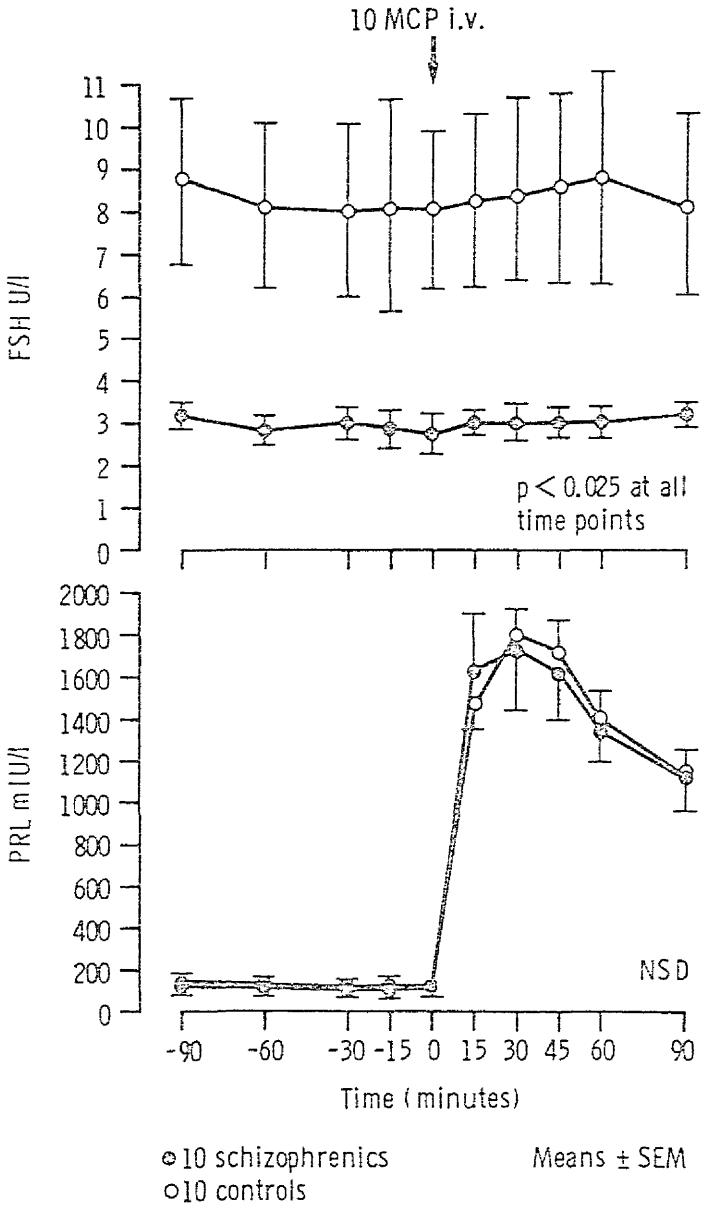
Serial LH, FSH and PRL in 3 chronic schizophrenics on two occasions o———o unmedicated and o----o after 6 months of neuroleptic medication as detailed in Table 6.1.

different sampling times, to assess PRL secretion rhythm on this occasion. In two patients (A and C : Fig 6.1) there was an elevation of LH and FSH secretion on the second occasion, with the development of evidence of pulsatile LH secretion in one patient (patient A - Fig 6.1). However there was no evidence of the development of episodes of LH secretion with a greater than 30% increment above baseline (the criteria of Boyar et al, 1972) in this patient. In patient B's case there was no change in LH or FSH secretion whilst on neuroleptics and he continued to show a low flat gonadotrophin secretion profile.

Study (2). The results of this study are shown in Figs 6.2 and 6.3 and in tabulated form in Table 6.3.

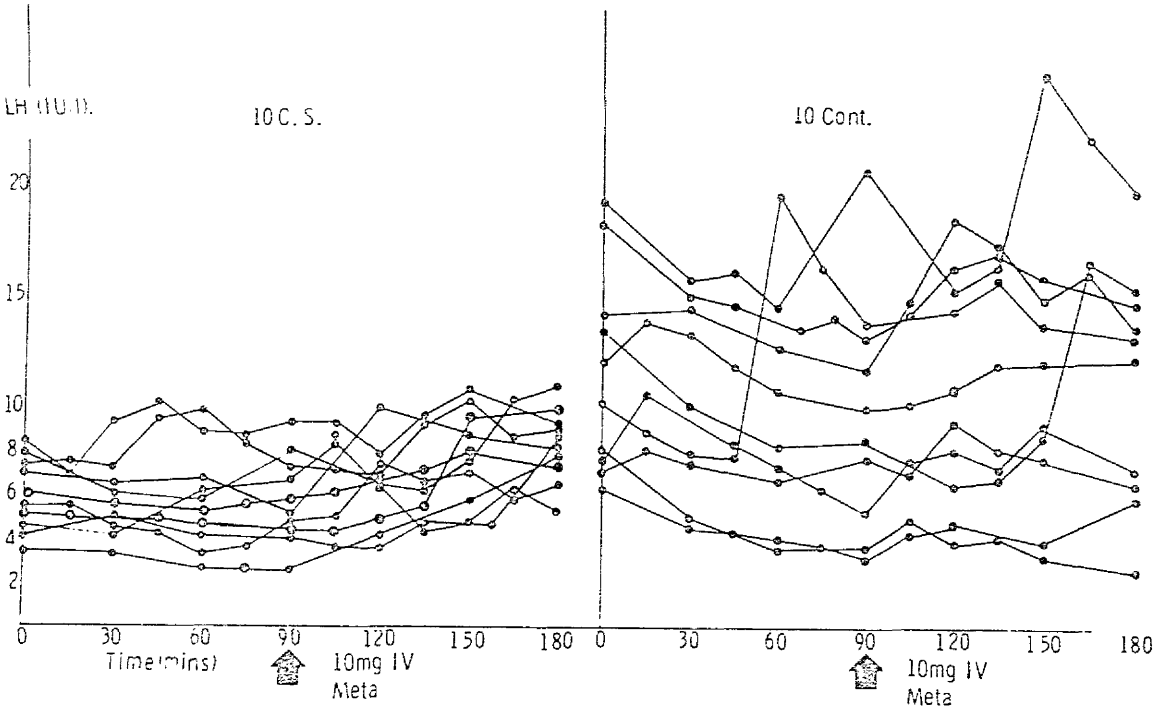
a) PRL. There was a large rapid and sustained rise in PRL secretion following MCP administration which was identical in both groups (Fig 6.2). There was no difference in the PRL secretion before or after MCP between the two groups at any time point or in terms of maximal PRL increment (Table 6.3).

b) LH. Mean LH was significantly lower in the chronic schizophrenic patients compared to controls prior to MCP administration. As can be seen from Fig 6.3 there was little spontaneous fluctuation of serial LH estimates from the chronic schizophrenics whereas there is a markedly different pattern in serial samples from the controls. The shorter sampling period of this study (90 mins) compared to that reported in chapter 4 (180 mins) and the reduced numbers studied (10 vs 10 and 20 vs 17 respectively) precludes an adequate statistical evaluation of this difference on this occasion.



Mean PRL and FSH levels before and after 10 mg MCP IV in 10 chronic schizophrenics and 10 controls FSH reduced ($p < 0.025$) at all time points and no change with MCP. PRL markedly elevated but no difference between the groups.

Fig 6.3



Serial LH estimations every 15 mins before and after 10 mg MCP IV in 10 controls and 10 chronic schizophrenics. LH reduced in schizophrenics vs controls before but not after MCP (see text).

TABLE 6.3

Group	LH IU/l			FSH U/l			PRL mIU/l	
	PRE MCP	POST MCP	+90min MCP	PRE MCP	POST MCP	+90min MCP	Basal	Peak POST MCP
Controls n=10	10.6 ±1.3 *	11.2 ±1.7	10.8 ±1.7	8.2 ±1.9 **	8.4 ±2.0 **	7.9 ±2.1 **	122 ±12	1854 ±164
Chronic Schizophrenics n=10	5.9 ±0.6	7.3 ±0.5	8.0 ±0.5 *	2.9 ±0.2	3.1 ±0.3	3.0 ±0.3	139 ±15	1872 ±227

LH, FSH and PRL levels before and after 10 mg IV MCP in 10 chronic schizophrenics and 10 controls. Values as means ± SEM. LH: reduced in chronic schizophrenics compared to controls prior to MCP * $p < 0.05$. Significant (* $p < 0.05$) rise post MCP in chronic schizophrenics. FSH: significant reduction (** $p < 0.01$) in chronic schizophrenics with no changes post MCP. Large rise in PRL, equal in both groups.

TABLE 6.4

Group	PRL m IU/l		LH IU/l		FSH U/l	
	Initial Week 0.	Mean Weeks 3,4,5.	Initial Week 0.	Mean Weeks 3,4,5.	Initial Week 0.	Mean Weeks 3,4,5.
Male acute schizophrenics n=22	257 ±50	590 ±64	7.6 ±0.7	6.5 ±0.4	2.1 ±0.2	2.2 ±0.3
Male aged matched controls n=11	234 ±23	-	6.1 ±0.5	-	2.4 ±0.5	-
Female acute schizophrenics n=14	955 ±364	1648 ±268 (n=12)	-	-	-	-

PRL, LH and FSH levels prior to and during weeks 3, 4 and 5 of oral flupenthixol therapy in 22 male and 14 female acute schizophrenics. Large rise in PRL secretion but no change in LH or FSH secretion. No difference between male patients and age-matched controls in any measure.

After MCP administration there was no difference in the mean LH level compared to the mean pre-MCP level in the controls nor was any difference at any particular time point noted (for example at the 180 minute time point). By contrast there was a statistically significant rise in LH level in the chronic schizophrenic group. The LH level rose from 5.9 ± 1.8 (before MCP) to 7.3 ± 1.6 (after MCP) (IU/l : means ± SEM) a difference that was significant at the $p < 0.05$ level. This difference was more marked at the 90 minute post MCP point when the LH level was 8.0 ± 1.6 ($p < 0.05$ compared with the mean pre-MCP level). The difference in LH levels between chronic schizophrenics and controls which was evident and significant prior to MCP administration disappeared following MCP administration (see Table 6.3).

c) FSH. FSH was significantly reduced in the chronic schizophrenic group compared to controls ($p < 0.05$). Neither group showed much fluctuation in serial FSH estimates. There was no change in FSH secretion following MCP administration in either group (see Fig 6.2) such that the significant difference in FSH between the groups was still evident after MCP administration.

Study (3). The results of this study are complex and only those with direct relevance to the effects of drugs on APHS in schizophrenia will be described in any detail. Basal levels of LH, FSH, PRL, GH and oestradiol from the male patients were discussed in chapter 4. Briefly the results of the clinical study showed:-

1) The dosage (titrated to produce an equal amount of sedation in each patient) varied widely from 1 - 48 mg of flupenthixol/day.

2) Positive symptoms (see chapter 3 section 2.1 for definition) were the most prominent symptoms and they showed a striking improvement throughout the period of the trial. There was no clearcut change in the infrequent negative symptoms.

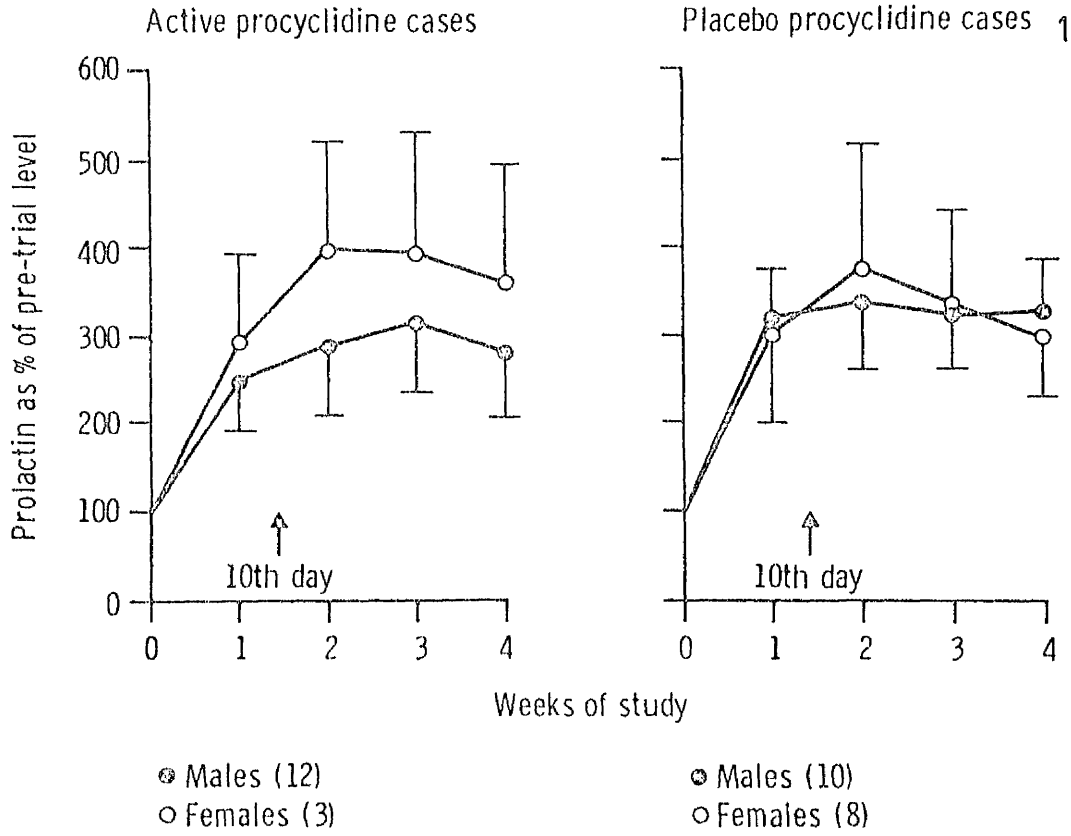
3) The addition of procyclidine to 16 of the patients on day 10 was associated with a temporary worsening of psychotic symptoms. There was in addition a reduced amount of total sleep time, but these two changes did not appear to be statistically related.

4) Extrapyramidal effects developed rapidly following the introduction of neuroleptics and increased throughout the course of the study. The introduction of procyclidine prevented further increases in extrapyramidal effects but did not abolish those already present.

As regards the laboratory measures and their relationship with clinical measures the following findings were demonstrated (Table 6.4):

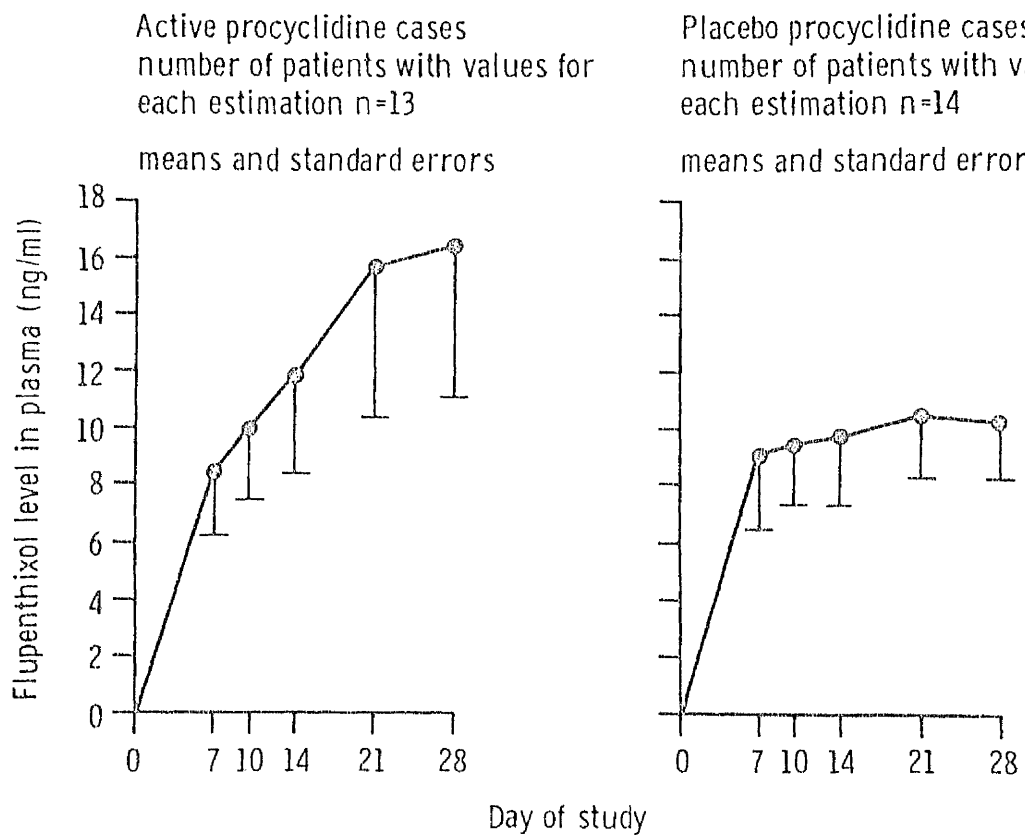
1) There was a significant rise in PRL secretion following neuroleptic administration which rose to a plateau after 14 days of therapy. Some female patients had very high levels of PRL before commencing the study probably due to prior neuroleptic medication. These changes are depicted in Fig 6.4. The mean PRL level of weeks 3, 4, 5 of the female patients was considerably higher than that of the male patients which was not entirely due to the slightly higher mean steady state dose in the females

FIG 6.4



PRL as a percentage of pre-trial level during 4 weeks of oral flupenthixol therapy in male (22) and female (11) acute schizophrenics. Large rises in PRL secretion to a plateau level. No effect of added procyclidine or placebo. Values as means ± SEM.

FIG 6.5



Serial flupenthixol levels in 27 acute schizophrenics during 4 weeks of oral flupenthixol therapy. Large rise as shown. No effect of procyclidine addition on day 10 and no significant differences between the groups.

(Table 6.4).

2) LH and FSH levels did not show any change with neuroleptic medication in the male schizophrenics. Furthermore LH and FSH levels were not different from an age - sex matched control group (Table 6.4).

3) The addition of procyclidine on day 10 did not affect either PRL levels (Fig 6.4) or flupenthixol levels (fig 6.5).

4) A significant relationship was found between the plateau level of flupenthixol (mean weeks 2, 3 and 4) and plateau level of PRL (mean weeks 2, 3 and 4) in both females ($p < 0.01$) and males ($p < 0.01$) and between steady state dose of flupenthixol and plateau levels of PRL in females ($p < 0.001$) but not in males. Among the males one patient had a steady state dose of 48 mg/day, the mean steady state dose of the whole sample being 8.3 mg/day. With this patient included the relationship between flupenthixol and plateau PRL was not statistically significant. If this patient is excluded the relationship between steady state flupenthixol dose and plateau PRL levels was significant at the 0.01 level.

5) The relationship between plasma level of flupenthixol and antipsychotic effect appears to be curvilinear. This relationship is not significant using polynomial regression but when the sample is placed in arbitrary categories a relationship which is significant when tested by unifed analysis of variance by ranks test is found.

6) No significant relationships were established between PRL levels and either antipsychotic or extrapyramidal effects. The

mixed sex and very variable pre-trial neuroleptic experience of the patients necessitate a subdivision of the sample into groups too small for meaningful graphical representation.

DISCUSSION

In brief, the results of these studies on the effects of neuroleptics on APHS in schizophrenia show that acute or chronic administration of DA blocking drugs is associated with some evidence of elevation of low gonadotrophin secretion in chronic schizophrenia. Despite close inter-relationships between PRL secretion and neuroleptic dose and serum level, neuroleptic administration to acute schizophrenics is not associated with changes in APHS that are likely to be clinically relevant or useful.

Looking at the results from chronic schizophrenics first, it was found that six months of oral neuroleptic therapy was associated with an elevation of FSH and LH secretion in 2 out of 3 patients. Acute DA blockade with metaclopramide was associated with a significant elevation in LH in the chronic schizophrenic group but not significant change in FSH or significant difference in the PRL response. These results are of potential interest since they can be taken as evidence that the mechanism of low gonadotrophin secretion in chronic schizophrenia may be increased dopaminergic tone.

These results to some extent confirm the findings of Brambilla et al (1975) who reported that one month's therapy with haloperidol to a group of chronic schizophrenic patients restored normal level of LH, FSH and testosterone secretion. It would

appear that an elevation of all the measured hormones took place in all of their cases. The reason for the non-response in one of the patients in the present study is not clear. While LH levels rose to a greater or lesser extent following acute DA blockade in all of the chronic schizophrenics, there was no change in FSH secretion which remained significantly below that of the control population.

These results should be considered in the light of the recent evidence conerning dopaminergic influences on gonadotrophin secretion which was discussed fully in chapter 2 (1.3). To review the data briefly, while it is more or less established that acute or chronic DA blockade does not induce any change in gonadotrophin secretion in normal individuals, the infusion of DA markedly reduces LH secretion but does not alter FSH secretion. Apparently therefore it is important when studying the effect of dopaminergic drugs on gonadotrophin secretion to consider the type and length of drug administration and whether normal or abnormal hormonal levels are being considered. It seems from the above data that DA influences on FSH are of minor importance. However it may be the case that due to differences in feedback effects (see below) FSH is influenced by only long term administration of dopaminergic drugs and perhaps only when the FSH level is abnormal for some reason (e.g. Brambilla's study, 1976).

These considerations are also relevant to the discussion of the study of Quigley et al (1980) who found that the administration of 10 mg MCP IV was associated with an elevation of LH, but not FSH, in female patients with hypothalamic hypogonadotrophic

amenorrhoea. They found that MCP administration had no effect on gonadotrophin secretion in normal subjects (confirmed in the present study). It would be interesting to study the effect of longer term DA blocking therapy on gonadotrophin secretion in the type of patients described by Quigley et al (1980) specifically to observe if any change occurred in FSH secretion under this circumstance as appears to be the case in chronic schizophrenia (Brambilla et al, 1975). A major difference however between the present study and that of Quigley et al (1980) is that in the latter case the PRL levels of the patient group were subnormal supporting the presence of increased dopaminergic tone as a cause of the aberrant LH levels whereas in the schizophrenic group PRL levels were normal. It is conceivable that in the case of the schizophrenic group the illness is of such long standing that feedback mechanisms operate to return PRL, but not gonadotrophins, to normal. Such a process has been observed in rats (Gudelsky et al, 1976) : PRL reversed to normal but LH fell when DA turnover in the hypothalamus was high. However to establish this process in schizophrenia requires intensive investigation. PRL levels also appear normal in acute schizophrenia (this study and others) and so these putative feedback changes in hormonal levels must be complicated.

As alluded to above these results are of potential interest since they give evidence of increased dopaminergic tone in some patients with schizophrenia. This is of interest since this is evidence which supports the DA hypothesis of schizophrenia which so far has rested only on indirect pharmacological evidence and on post-mortem biochemistry (both methods being fraught with

difficulties of measurement and interpretation). This hormonal finding is in living patients who have undoubted schizophrenia. These results (as seems to be the case with all research) only raise more questions. One of these is why these effects are seen only to patients with chronic schizophrenia (patients with acute schizophrenia have normal gonadotrophin secretion and no change induced by neuroleptics). Chronic schizophrenics as a general rule have an inadequate therapeutic response to neuroleptics (Letemendia and Harris, 1967) and this almost certainly applies to the group of patients studied here. For example the three patients in the present study who were placed on neuroleptic treatment for six months showed no clinical amelioration of their disabling symptoms. Thus the abnormalities detected here (i.e. low LH with loss of pulsatility (see chapter 4) and its correction by neuroleptics) supports the DA hypothesis of schizophrenia. It is however found in a group of patients who have either never had neuroleptics or in whom neuroleptics were discontinued at least to some extent because of lack of response. These patients therefore cannot be said to have a clinical response to neuroleptics which supports any hypothesis based on overactivity of dopamine neurotransmission. Difficulties are also associated with the finding of increased post-synaptic DA receptors in schizophrenia (Owen et al, 1978) which was detected in a series of brains mostly from patients with chronic schizophrenia. However in a subsequent study it was found that the increased DA receptors correlated with the positive, but not negative, symptoms of these patients (Crow et al, 1980). Thus there are

indications of two separate pathophysiological processes in schizophrenia which may run in parallel viz 1) increased dopaminergic neurotransmission (responsible for acute drug responsive symptoms) and 2) an other slower, perhaps destructive, process (responsible for chronic drug unresponsive symptoms). The present study and the PM study indicate that DA overactivity may be present and detectable in drug unresponsive chronic schizophrenics. To clarify this position further a study looking at the hormonal effects of acute DA blockade with IV MCP in a variety of acute and subacute cases of schizophrenia is underway.

Further aspects of the interpretation of the present results to consider in the light of the broadly comparable result of Quigley et al (1980) in patients with stress induced amenorrhoea are a) that increased dopaminergic tone may be a pathophysiological counterpart of stress and that this operates in the chronic schizophrenic group and b) that increased dopaminergic tone may be the final common process of any form of hypogonadism. These questions are not easy to answer due to the paucity of data but to take them in turn:- a) this appears an unlikely idea since:- 1) most research indicates no role for DA in the mediation of stress 2) the chronic schizophrenics did not appear "stressed" and 3) the controls used had neurotic illness and were subjected to the same technical manipulations. Point b) cannot be refuted or confirmed since comparable studies on a variety of causes of hypogonadism have not been performed (however, theoretically, DA tone is likely to be high, rather than low, in patients with hyperprolactinaemic hypogonadsim).

Finally the PRL response to IV MCP was identical in the chronic schizophrenic group compared to the control group (see Table 6.4). This confirms the work of Gruen et al (1978) and indicates that an abnormality of the lactotrophe and/or pituitary DA receptors is unlikely in cases of schizophrenia. The possible reasons for the normality of the PRL response to neuroleptics but the blunted response to TRH (Chapter 5) in comparable chronic schizophrenics will be discussed in chapter 8.

Turning to the results from the study on acute schizophrenics (Study 3), the following points are evident and important. Flupenthixol and PRL levels rose during treatment and significant relationships were established between them and daily flupenthixol dose. The PRL response to flupenthixol was very variable even when male and female patients are separated, and did not relate to the clinical effects or side effects of neuroleptics nor was it affected by anticholinergic medication. Gonadotrophin levels were not significantly affected by neuroleptics.

Similar relationships between neuroleptic dose, plasma level of neuroleptic and prolactin level were established by Rao et al (1980) studying females with chronic schizophrenia on stable doses of haloperidol but in two series of schizophrenic patients treated on an acute basis with chlorpromazine (Kolakowska et al, 1975; Wiles et al, 1976; Kolakowska et al, 1979) a significant relationship between plasma level of neuroleptics and prolactin but no relationship with dosage was found. In these studies the numbers were relatively small and the dosage of

chlorpromazine was determined by clinical judgement.

It is of interest that there is a relationship between the dose of neuroleptic, the blood level obtained and the consequent PRL secretion because it implies that PRL secretion can be used as an index of neuroleptic (and probably DA blocking) activity. There are however several important limitations to the use of PRL secretion as a marker of drug ingestion. The most notable of these is that these relationships are only statistically significant in large groups of patients and that individual patients fairly frequently exhibit large variations (e.g. patients given a very high dose of drug exhibit low blood levels and low PRL secretion and vice versa). The use of PRL secretion as an index of neuroleptic drug compliance is also problematical since it has been shown that long term drug therapy is associated with a tolerance to the PRL elevating effects of neuroleptics and that in many cases, particularly in elderly men, PRL levels return to the normal range (de Rivera et al, 1976; Huws and Groom, 1977).

In the present study no significant relationships were demonstrated between PRL and either antipsychotic effects or extrapyramidal side effects. Positive relationships between the antipsychotic effect of neuroleptics and the associated elevation of PRL have previously been demonstrated by some workers (Meltzer and Fang, 1976; Langer et al, 1977; Cotes et al, 1978) but not by others (Kolakowska et al, 1975; Kolakowska et al, 1979). It is clear from all these studies that the relationship is not strong (i.e. many patients with high PRL levels frequently exhibit

minimal or no clinical response and vice versa) and the effect is only obvious at low doses of neuroleptics as a ceiling effect develops (Gruen et al, 1978). A time-lag between the rapid elevation of PRL secretion and the slower anti-psychotic effects of neuroleptics was noted in a previous study (Cotes et al, 1978) but was not so marked in the present study.

The relationship between PRL level and extrapyramidal side effects has been less widely studied. Rao et al (1980) did find the two to be significantly related but was studying female chronic patients who had been on a stable dose of neuroleptic for 6 months. In the study of Wiles et al (1976) there is a non-significant tendency for extrapyramidal side effects to be associated with higher prolactin levels and a similar tendency significant in some but not all comparisons was found in the study of Kolakowska et al (1979). These two studies like the present one include patient samples of variable age, sex and past neuroleptic experience and it may be that these features have tended to obscure a relationship between extrapyramidal effects and prolactin which might otherwise have been found.

In this study the administration of anticholinergic drugs did not significantly affect either flupenthixol blood levels or PRL levels. There are several conflicting reports in the literature on the effects of anticholinergics on neuroleptic blood levels probably resulting from differences in design and methodology (see Simpson et al, 1980, for review). Whatever the true nature of the relationship between these two types of drugs it is clear that in the present study the effects of

procyclidine upon the extrapyramidal and mental state ratings (see clinical results page 181) must result from a mechanism other than reduction of neuroleptic blood levels.

The question of the effect upon PRL levels of the addition of anticholinergics to the drug regime of patients on neuroleptics has been less widely studied though as disputed. De Rivera et al (1976) found that anticholinergics had no effect upon PRL levels in patients treated acutely with neuroleptics but that in patients on chronic neuroleptic treatment those on concurrent anticholinergics had higher PRL levels than those without anticholinergics.

A similar study concluded that an effect of anticholinergics on PRL secretion was only obvious at high doses of anticholinergics and low doses of neuroleptics (Halbreich et al, 1980). Lal et al (1979) but not Halbreich et al (1980) found an increase in the PRL elevation induced by neuroleptics following acute anticholinergic administration to normal volunteers. The findings in acutely treated patients in this study are consistent with the earlier findings of de Rivera et al in acute patients.

The male patients in this acute study had mean gonadotrophin (LH and FSH) levels which were exactly comparable with an age-matched control group (results shown in section 3, chapter 4) confirming the indication that gonadotrophin control is not disturbed in acute schizophrenics. Furthermore neuroleptic administration did not significantly alter gonadotrophin secretion in these patients (although in the 3 patients with the lowest pre-drug levels rose into the normal range after 4 weeks of flupenthixol). The contrast with chronic schizophrenics and

implications of this finding were discussed above.

In conclusion these studies on the effects of neuroleptics on anterior pituitary secretion in schizophrenics reveal that the most marked effect is an elevation of PRL secretion but that this does not prove a useful clinical measure or a discriminating feature of schizophrenia. However a most interesting effect has been noted, namely that low gonadotrophins of chronic schizophrenia are at least partly reversed by acute and chronic neuroleptic administration, which may prove to be a valuable research tool in the understanding of the pathophysiological process(es) of schizophrenia.

CHAPTER 7

Effect of a dopamine agonist on hormonal and clinical state of acute and chronic schizophrenics

INTRODUCTION

Since the DA overactivity hypothesis of schizophrenia was first postulated, researchers have been interested in testing it by examining the effects of DA agonists in schizophrenic patients. Particular attention has been paid to the clinical effects of these drugs but evidence of altered DA receptor sensitivity has also been sought.

An outline of the pharmacology of various DA agonists was given in Chapter 2. As was mentioned in that section (1.3) there are several reservations about the use of amphetamines and L-DOPA as DA agonists and so the choice lies between apomorphine (APO), a synthetic derivative of morphine with direct DA receptor agonist properties (and no opiate-like activity), and the ergot derivatives. The latter drugs, while more specifically agonists at DA2 receptors (the predominant DA receptor in the pituitary), have more activity than APO on other neurotransmitter systems (notably 5HT). In addition there are problems associated with the administration of the ergot derivatives in that they can only be given orally and therefore their pharmacological effects are of gradual onset and are prolonged. These considerations lead to the choice of APO as the drug to study the hormonal and clinical effects of dopamine agonists in schizophrenia. APO can be administered s.c. with good, rapid absorption into blood and the CNS and is rapidly metabolised in the liver.

The hormonal effects of APO have been extensively documented during the past decade. It has been shown that APO induces a rapid and large elevation of GH secretion in man in doses of 0.25-1.5 mg s.c. (Lal et al, 1973). Doses below 0.25 mg are without any effect and doses above 1.5 g cannot be given as the frequency of side effects (nausea, vomiting) is too high. Cleghorn et al (1982) have shown a stepwise dose-response curve with increasing doses of APO in this range. It has been shown that repeated doses of 0.75 mg of APO produce reproducible response within individuals. There is a large difference in the magnitude of the GH increment after APO between individuals (Rotrosen et al, 1979). Ettigi et al (1975) reported some of the factors which are responsible for this variable response. These include reductions in the response induced by high oestrogen levels, by elevated glucose levels and by alterations in posture. It is clear that there is also a large inherent inter-individual variability. The GH response to APO is consistently blocked by neuroleptics, but not by other neuroactive drugs (Lal et al, 1977) which indicates that DA receptors are involved in the mediation of this response. However the site of this mediation (i.e. within the CNS or the periphery) is still unclear despite intensive research but is most probably at the hypothalamic level.

APO also suppresses PRL secretion and this effect is seen in a variety of circumstances e.g. pituitary cell culture lines, animals, normal subjects and hyperprolactinaemia (Johnstone and Ferrier, 1980 for review). The effect is much more striking when PRL levels are high, through any cause, and the effect is minimal

when PRL levels are low as there appears to be a PRL level below which even massive doses of the drug do not further depress PRL secretion. The effect of preceeding neuroleptic drug therapy in humans on PRL suppression by APO has not been clarified. In animals evidence of functional supersensitivity of this response can be detected after neuroleptics have been withdrawn for a few days (Lal et al, 1978) (despite the fact that there is no increased sensitivity of pituitary DA receptors at this time (Friend et al, 1978)).

It seems clear from the literature that the administration of APO is not associated with any change in LH, FSH, ACTH, TSH, or cortisol secretion (Checkley, 1980). The GH increment after APO is blunted in a substantial proportion of chronic schizophrenic patients (Rotrosen et al, 1979). The significance of this findings has not been established. It has been suggested that prolonged neuroleptic therapy leads to this blunting of response (Ettigi et al, 1976) but this has been disputed by other workers (Cleghorn et al, 1982). It would appear from the cumulative data of several studies (see Table 2.4, chapter 2) that the mean GH increment after APO is normal in acute schizophrenia (Ettigi et al, 1976; Rotrosen et al, 1979). However all these studies have reported that the GH response in acute schizophrenics is very variable and that there are many outliers above and below the control range. Cleghorn et al (1982) suggest that those patients in the process of relapsing are those who exhibit the enhanced response. This requires confirmation and the possible confounding effects of the short withdrawal time from neuroleptic

drugs (often as little as 7 days) needsto be taken into account. For these reasons a study looking at the hormonal response to APO in acute and chronic schizophrenics in male only, fasting patients was carried out. These patients had been drug free for at least six months. An appropriate control group were also studied.

The clinical effects of apomorphine in schizophrenia have recently provoked much interest. Before the discovery of the antipsychotic properties of the phenothiazine drugs, APO was administered to schizophrenic patients when temporary relief of psychotic symptoms was necessary. Case reports in the older literature describe its beneficial action as a short acting "tranquilliser" (Feldman et al, 1945). Sedation was thought to be the principal mode of action, but recent preclinical observations suggest that APO may exert any antipsychotic efficacy via a reduction in DA neurotransmission. DA agonists at low doses appear to activate presynaptic DA receptors preferentially (Meltzer, 1980 for review) such that DA synthesis and release is inhibited. Therefore as opposed to predicting (according to the increased DA receptor theory of schizophrenia) that APO should exacerbate psychotic symptoms it is conceivable that exactly the opposite effect might be noted. There have recently been some clinical observations to support this notion. Tamminga et al (1978) reported significant reductions in the psychotic symptoms of a group of 18 schizophrenic patients following 3 mg of APO s.c. It should be noted that these patients were also on neuroleptic medication at the time of the study.

Corsini et al (1977) reported a similar effect in about half a large group of unmedicated schizophrenic patients (although have subsequently modified this therapeutic claim to apply mostly to schizo-affective patients (personal communication)). Meltzer et al (reported in Meltzer, 1980) noted a striking therapeutic effect in a subgroup of schizophrenic patients. However none of these studies have performed blind clinical ratings and acute schizophrenics have not been studied. Furthermore if this effect was a real or marked one, then it should also be found with ergot derivatives which have a similar pharmacological profile. However it would appear that bromocriptine and other ergolines, are not associated with therapeutic efficacy and may exacerbate psychotic symptoms (Tamminga et al, 1979). Accordingly a study of the clinical effects of APO, blindly rated in well diagnosed acute and chronic schizophrenic patients to supplement the hormonal studies seemed appropriate. Recent evidence suggests that spontaneous eye blink rates may be an indicator of central DA activity. Blink rates are decreased in Parkinson's disease. APO increases blink rates in monkeys in a dose-related fashion and this dose response curve shifts to the left after three weeks haloperidol treatment (Karson et al, 1981).

It has been reported that blink rates are increased in some schizophrenic patients (Stevens, 1978) and show a decrease in such patients following neuroleptic therapy (Karson et al, 1981). It has recently been reported that the change in eye blink rates caused by haloperidol therapy corresponds to a change in the thought disturbance syndrome (Karson et al, 1982).

The effects of APO on clinical side effects, symptoms and eye blink rates in acute and chronic schizophrenics and controls are reported here along with a description of concommitant hormonal changes.

MATERIAL AND METHODS

Patients studied. Three groups of patients were studied:-

a) 15 male chronic schizophrenic patients were selected according to the criteria of age, diagnosis, health and drug free status outlined in chapter 3 (section 1.1) and as described in chapter 4 (section 2).

b) 15 male acute schizophrenic patients were selected according to the criteria outlined in chapter 3 (section 1.2) and as described in chapter 4 (section 2).

c) 10 male controls were studied. Normal laboratory and medical staff volunteers were chosen to age match the acute schizophrenic group (b, above).

Clinical characteristics of the patients studied in terms of age, length of illness, drug history and mean positive and negative symptom scores (rated on the Krawiecka scale (see chapter 3, section 2.1)) are shown in Table 7.1.

Experimental Protocol

The following protocol was carried out in all patients and is schematically represented in Fig 7.1.

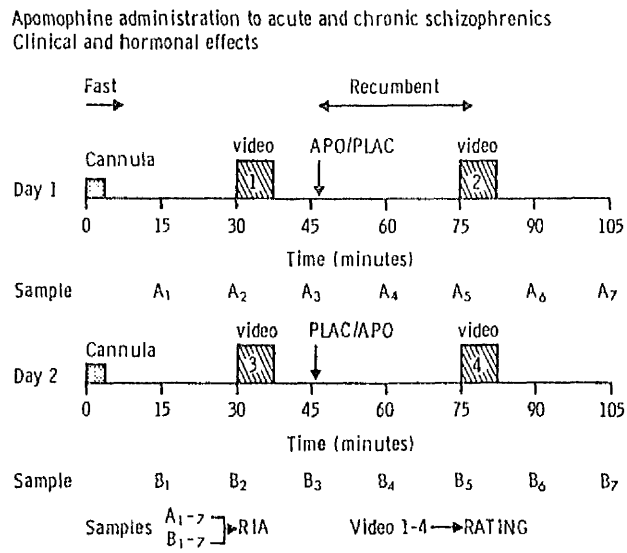
1) All patients were studied after a 10 hour fast. A butterfly cannula was first inserted between 8 and 8.15 a.m. and left in situ for 15 - 30 mins prior to the first sampling.

TABLE 7.1

Group	n	Age (yrs)	Age at onset of illness (yrs)	Positive symptoms*	Negative symptoms*	Drug history
Controls	10	27 ± 2.0 (20-36)	-	-	-	None
Acute schizophrenics	15	30.1 ± 2.0 (18-41)	27 ± 2.0 (16-49)	6 (2-10)	1 (0-5)	9 patients never received neuroleptics. 6 patients stopped neuroleptics for 1/12 - 3 yrs (mean 10/12)
Chronic schizophrenics	15	62 ± 2.0 (39-70)	25 ± 1.1 (15-30)	3 (0-9)	3 (0-7)	5 patients never received neuroleptics 10 stopped 1 - 8 yrs previously (mean 3 yrs). 1 patient on Brufen. 1 patient on Diuretics

Clinical data in terms of age, age at onset of illness, positive and negative symptom ratings and drug histories of groups studies with apomorphine. Values as means ± SEM - range given in parentheses.
* values as modes.

FIG 7.1



Schematic representation of apomorphine study. Videos were made 15 mins before and 30 mins after apomorphine (APO) or placebo (PLAC). Schedule of APO/PLAC was randomised. Samples taken as indicated by A1 - A7 and B1 - B7.

2) Two venous samples were then taken at 15 min intervals.

3) A semi-standardised interview was carried out by a consultant psychiatrist and recorded on videotape. In the case of controls a short perception test was carried out at this time (listening to a tape recording and listing the frequency of key words).

4) A further venous sample was taken followed by the administration of 1 ml s.c. of either 0.75 mg APO or identical vehicle into the upper arm (the order of injections being determined by a random schedule).

5) The patient then lay recumbent for 30 mins during which time 2 further venous samples were taken at 15 min intervals. An assessment of side effects experienced by the subjects (drowsiness, nausea, vomiting, yawning) was made during this time on a simple absent/mild/moderate/severe scale by means of a mixture of objective and subjective observations.

6) A further videotaped interview was then made lasting approximately 5 - 10 mins. The perception test was repeated on the controls.

7) Two further venous samples were obtained at +45 mins and +60 mins post APO/placebo.

8) On the following day the procedure was repeated giving either vehicle or APO whichever had not been administered the previous day.

9) Sera was prepared from venous samples as outlined in chapter 3 (section 3). Samples were assayed for LH, PRL, GH and oestradiol by the methods outlined in chapter 3 (section 4.3).

10) Videotapes were rated according to the scheme devised by Krawiecka et al (chapter 3, section 2.1) by two separate psychiatrists who were not aware of the order of injections (or of the other's rating). There was a high degree of concordance between these ratings.

11) Eye blink rates were measured by two investigators from the videotapes by counting blinks (with the aid of a computer programme) in the 2nd and 5th minute of the tape.

A close correlation was found between the ratings of these two investigators and between the two different test minutes such that the mean of all four figures is included for the purpose of the present analysis.

Radioimmunoassays

1) GH was estimated by RIA by the method given in Chapter 3. GH was measured in 8 separate assays each of which contained samples from each treatment group, but all samples from an individual patient were measured in the same assay. Dilution steps were performed if the result exceeded 12 mU/L. The co-efficient of variation for high, medium and low quality controls was 14%, 8% and 10% respectively.

2) PRL and LH were estimated by RIA by the method given in Chapter 3. PRL and LH were measured in 5 separate assays which conformed to the principles laid down above. The co-efficient of variation for high, medium and low quality controls for PRL and LH respectively were 12%, 10% : 8%, 7% : 10%, 7%.

3) Oestradiol was estimated in the pre-placebo sample according to the method set out in chapter 3 in two assays

containing samples from patients and controls (which were subject to stringent external quality control).

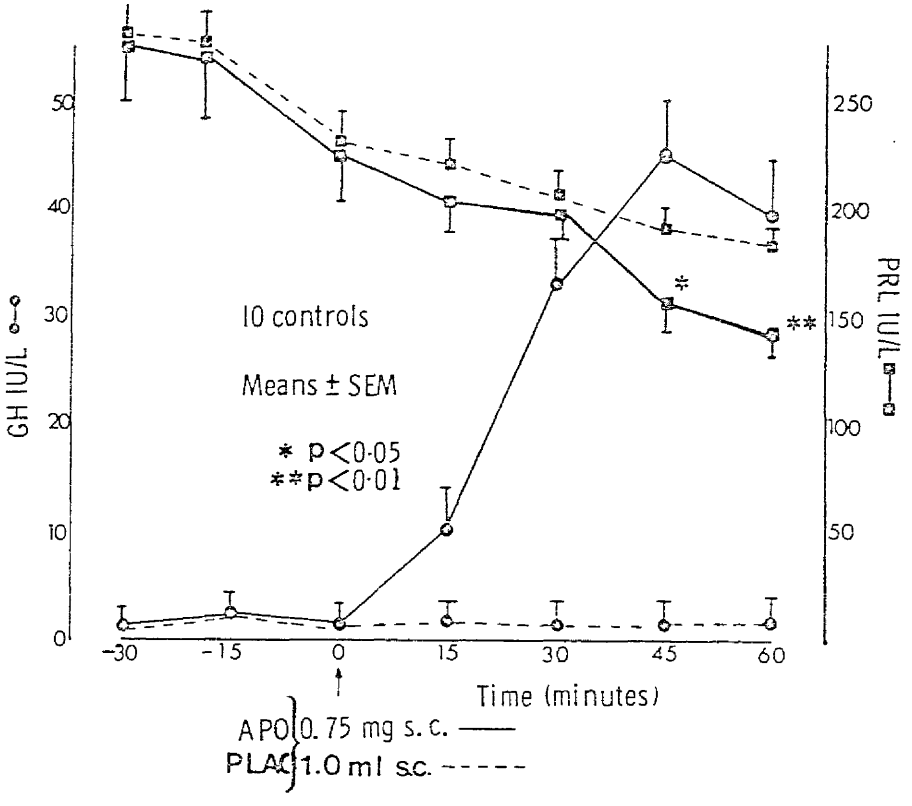
RESULTS

A) Hormonal results

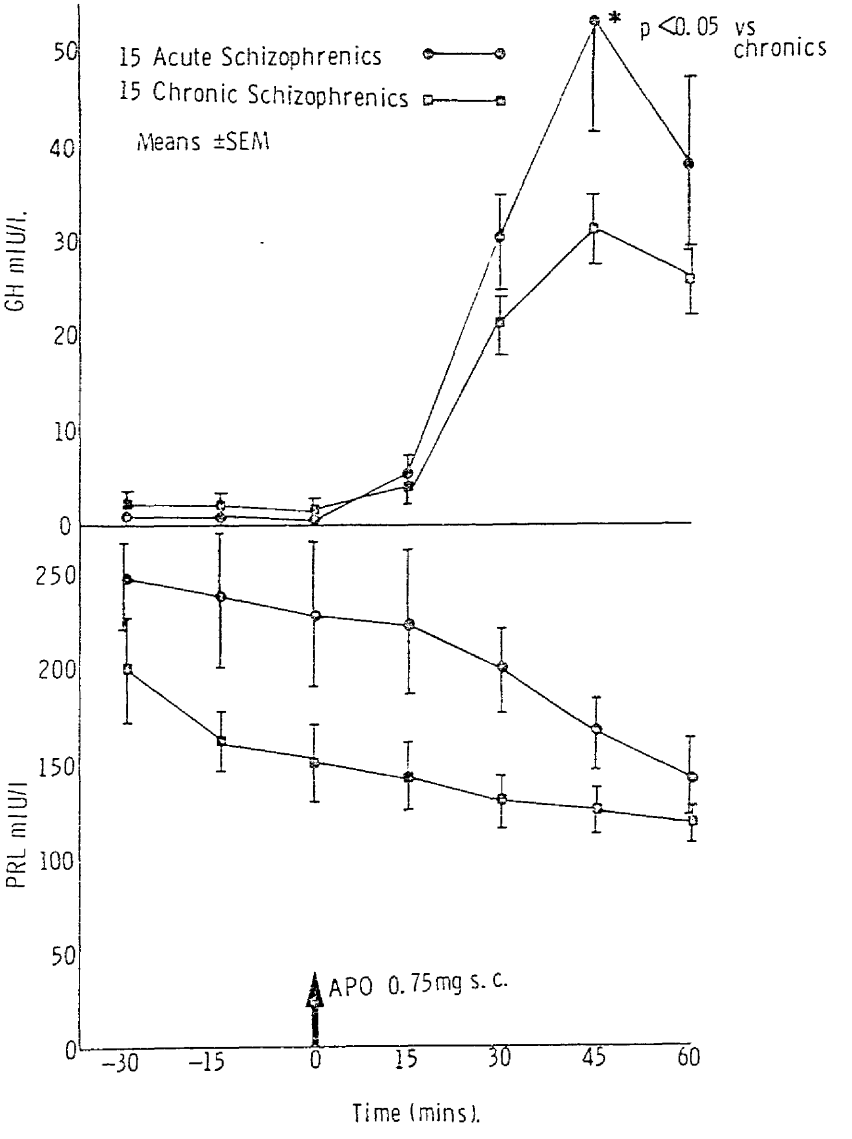
a) Results in normal subjects. The mean GH and PRL responses to APO in normal subjects are shown in Fig 7.2. It can be seen that there was a large, but variable, GH response with a mean peak increment at $+45^{1}$. The GH levels peaked at $+45^{1}$ post APO in samples from 8 of the 10 controls (one subject exhibited a GH peak at +30 minutes post APO and one subject at +60 minutes). PRL suppression post APO was a slower and less marked phenomenon. As discussed in chapter 2 (section 1.3 on hormonal rhythms) PRL levels tend to fall in the early post waking period of the morning and this effect was seen in all of the controls studied here. Thus there was no significant APO induced reduction in PRL secretion till +45 minutes post APO (see Fig 7.2 for significance levels). LH levels were unaffected by APO administration (Table 7.2).

b) Differences in hormonal responses of acute and chronic schizophrenics compared to controls. The results for the patient groups are depicted in Figs 7.3 and 7.4 and comparisons in Table 7.2. The timing of the changes in GH and PRL in schizophrenics was similar to those of controls (Fig 7.2).

1) GH. There was no difference in the mean baseline level of GH (mean of 3 pre-drug levels) between the groups studied (Table 7.2). A scattergram of peak GH increments after APO (max GH



GH and PRL secretion before and after APO or placebo in 10 controls. GH elevation significant post APO at all time points vs placebo. PRL secretion significantly lower at +45 and +60 minutes.



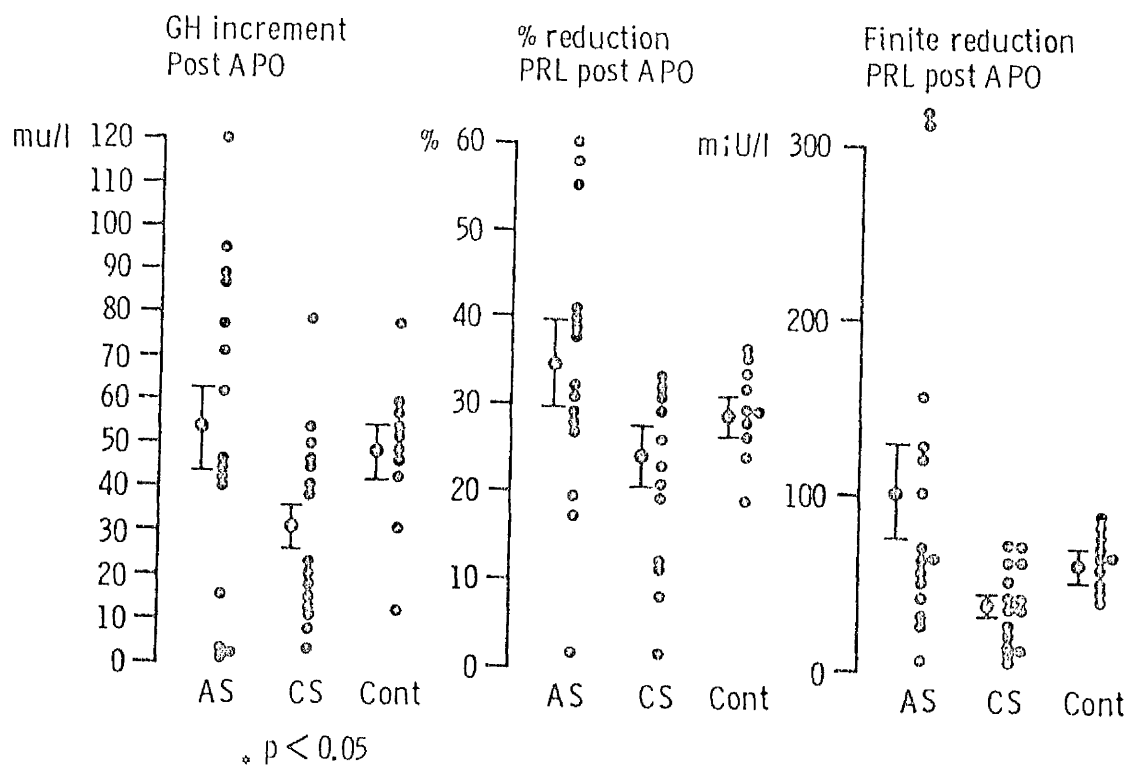
GH (upper panel) and PRL (lower panel) secretion post APO administration in 15 acute and 15 chronic schizophrenics. +45' GH secretion significantly higher in acute patients. No other significant differences.

TABLE 7.2

Group	GHmU/l		PRLmiU/l			LHIU/l		
	Basal	Increment (Peak-Basal)	Basal (Pre APO)	+60' (POST APO)	% Reduction	Basal (Pre APO)	+60' (POST APO)	E2 pmol/l
Controls n=10	1.1 ±0.3	48 ±6.4	225 ±14	159 ±9.6	29 ±2.4	5.5 ±0.5	5.4 ±0.3	60.1 ±9.5
Acute Schizophrenics n=15	14 ±0.2	53 ±9.4	275 ±38	170 ±18	34 ±4.2	5.9 ±0.8	5.7 ±0.7	52.1 ±7.3 (10)
Chronic Schizophrenics n=15	24 ±0.7	31 * ±5.0	158 * ±11	122 ±8.5	24 ±3.1	6.9 ±0.7	6.7 ±0.6	71.1 ±5.9

Basal GH and peak increment in GH (peak-basal), post APO, basal PRL + LH and at +60' post APO, % reduction in PRL and basal E_2 levels in 3 groups studies. Values as means ± SEM. * $p < 0.05$ vs acute schizophrenics and controls.

FIG 7.4



Scattergram of GH increment (peak-basal), % reduction and finite reduction (0' - +60') in PRL secretion in 15 acute schizophrenics (AS), 15 chronic schizophrenics (CS) and 10 controls (CONT).

level after APO - mean baseline level) is shown for the three groups in Fig 7.4. There was no significant difference between the acute schizophrenics and the controls but the chronic schizophrenic group's mean peak GH increment was reduced compared to both groups ($p < 0.05$).

It was noted that there was a very large variation in the post APO GH increment in the acute schizophrenic group (ranging from +1mlU/l to +120 mlU/l). This variability just failed to be statistically significantly greater than that of the control group ($f = 3.24$ (2,12) NS:5% level $f = 3.28$).

2) PRL levels. There was no difference in basal PRL levels between acute schizophrenics and controls but the levels were lower in the chronic schizophrenic group compared to either group above ($p < 0.05$). This no doubt reflects the greater age of this group (see chapter 4, page 133 and results (section C below).

There are several ways of assessing PRL suppression after APO. These include the percentage reduction at 60 minutes post APO compared to 0 minutes, the finite reduction in mIU/l at these two times and the finite reduction in mIU/l between these times less the finite reduction between these times on the occasion of placebo administration. The results for the first two of these methods of assessment for the three groups are shown in Fig 7.4 in scattergram form and the mean values for the third are detailed in the text.

It can be seen from Fig 7.4 that there was no significant difference between the groups in the PRL reduction following APO either in percentage terms or in terms of finite reduction in

mIU/l. As can be seen from Fig 7.4 there was a very large variation in the magnitude of PRL reduction in the acute schizophrenics. Analysis revealed that this increased variability related to the increased variability in the level of PRL prior to APO administration. There was a highly significant relationship between the reduction in PRL after APO and basal levels of PRL ($r = 0.91$ $n = 40$ $p < 0.001$ i.e the reduction was greatest where levels were higher and least when levels were low). There was no statistical relationship between the increment in GH after APO and PRL levels or suppression.

3) LH levels. Mean LH levels before and after APO and placebo are given for the three groups in Table 7.2. It can be seen that APO administration was not associated with any change in LH secretion.

4) Oestradiol levels in the three groups studies are shown in Table 7.2. As can be seen there was no statistical difference between the groups.

B) Clinical effects of apomorphine

a) Side effects. The frequency of side effects - drowsiness, nausea, vomiting and yawning - are shown for each group in Table 7.3.

It can be seen that there was a considerable variation in the frequency and severity of these effects, but there were no significant differences between the groups. Approximately a third of each group had no subjective or objective signs but a similar proportion felt quite unwell with severe nausea and/or

TABLE 7.3

Group		Sedation			Nauseo/Vomiting			Yawning		
		None	Mild/ Moderate	Marked	None	Mild/ Moderate	Marked	None	Mild/ Moderate	Marked
Controls n=10	n=	4	4	2	3	5	2	3	5	2
	%=	40	40	20	30	50	20	30	50	20
Acute Schizophrenics n=15	n=	5	7	3	7	4	4	10	4	1
	%=	33	47	20	47	27	27	66	27	7
Chronic Schizophrenics n=15	n=	9	5	1	9	4	2	10	2	3
	%=	60	33	7	60	27	13	66	14	20

Number of subjects exhibiting and frequency in each group of side effects following 0.75 mg APO s.c. in controls and acute and chronic schizophrenics. No significant differences.

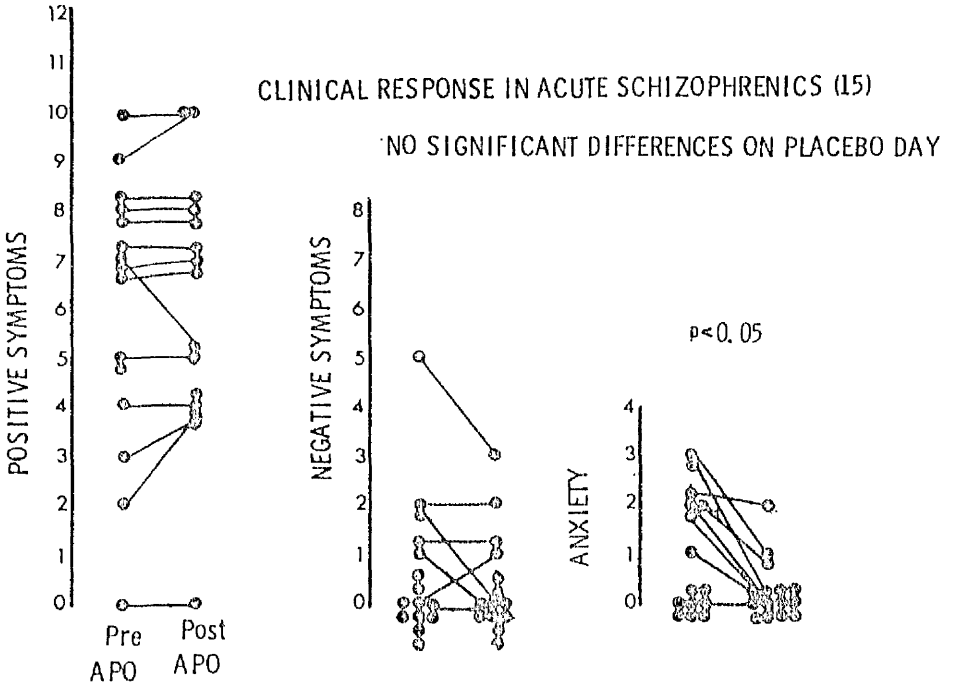
TABLE 7.5

Group		BLINK RATES (Blinks/min)			
		PRE PLACEBO	POST PLACEBO	PRE APOMORPHINE	POST APOMORPHINE
Acute Schizophrenics n=12	Mean	13.5	14.7	12.8	13.8
	SEM	1.2	1.7	1.2	1.5
	Range	5-20	4-23	5-18	4-20
	% Change	-	+ 7	-	+ 8
Chronic Schizophrenics n=13	Mean	12.5	14.3	12.1	13.2
	SEM	2.3	2.1	1.9	2.5
	Range	3-37	4-29	3-30	4-34
	% Change	-	+ 26	-	+ 12

Blink rates before and after placebo and apomorphine in 12 acute schizophrenics and 13 chronic schizophrenics. No significant group or apomorphine effects.

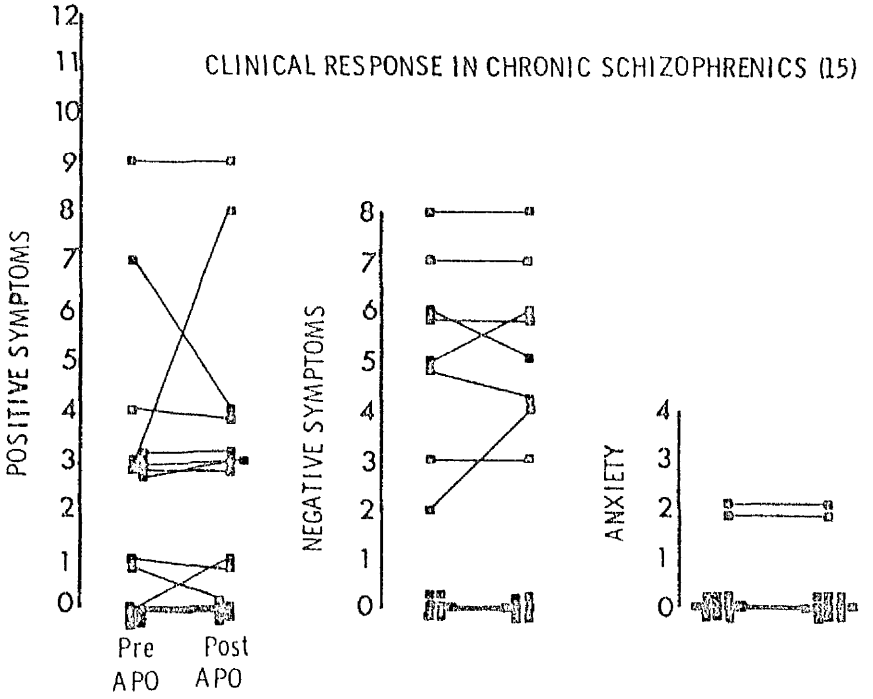
vomiting. In these latter cases the unpleasant feeling came on about 15 minutes after administration of APO but passed off rapidly usually by +30 minutes. It was found that there was no correlation between GH increments, PRL suppression and the frequency or severity of APO side effects.

b) Effect on clinical ratings. Clinical ratings before and after APO have been depicted in Fig 7.5 and Fig 7.6. Mean values are set out in Table 7.4. It can be seen that there was a large variation in the symptoms of these patients at the time of testing with positive symptoms (delusions, hallucinations, incoherence of speech and incongruity of affect) more prominent in the acute schizophrenic group than the chronic schizophrenic patients where negative symptoms (poverty of speech and flattening of affect) predominated. Non-specific features (anxiety, depression, retardation) were relatively uncommon in both groups. Table 7.4 shows that, while there was some change in some symptoms in a few patients, there was no significant change in any of the typically schizophrenic symptoms (i.e. positive or negative symptoms) following the APO injection. Positive symptoms improved in 6 out of 7 patients whose rating changed but the magnitude of this improvement was small. The improvement of positive symptoms was not frequent enough to be statistically significant ($p = 0.07$, Sign test). There was however a significant reduction in the anxiety ratings of the acute schizophrenic patients following APO ($p < 0.05$, Sign test). This was not due to a placebo effect of becoming accustomed to the videotaped interview since anxiety ratings on the pre- and post placebo occasion were



Positive and negative symptoms and anxiety ratings before and after APO (0.75 mg s.c.) in 15 acute schizophrenics. Reduction in anxiety significant at $p < 0.05$ level.

Fig. 7.6



Positive and negative symptoms and anxiety ratings before and after APO (0.75 mg s.c.) in 15 chronic schizophrenics. No significant differences.

TABLE 7.4

Symptoms	Mean rating Acute schizophrenics n=15		Mean rating Chronic schizophrenics n=15	
	PRE APO	POST APO	PRE APO	POST APO
Delusions	3	3	4	3
Hallucinations	2	2	1	1
Incongruity of affect	1	0	1	1
Incoherence of speech	1	1	1	1
Positive symptoms subscore	7	6	7	6
Flattening of affect	0	0	2	2
Poverty of speech	0	0	1	2
Negative symptoms subscore	0	0	3	4
Anxiety	1 *	0	0	0
Depression	0	0	0	0
Retardation	0	0	1	1
Total mean score	8	6	11	11

Clinical effect of APO in acute and chronic schizophrenia. Scores are means for each group on each symptom of the Krawiecka scale. Also shown are subscores for positive and negative symptoms (see text). * $p < 0.05$ (Sign test).

comparable with pre-APO anxiety ratings. It was found that there was no correlation between the sedation following APO and the reduction in anxiety.

c) Effect of APO on eye blink rates. Results for eye blink rate measurements are set out in Table 7.5. No data are available from controls. It was not possible to obtain results from 2 of the chronic schizophrenics and 3 of the acute schizophrenics for a variety of reasons (e.g. poor quality videos, hand in front of face etc.).

It was found (Table 7.5) that there was a wide variation in the eye blink rates within the acute and the chronic schizophrenic group. There was, however, high degree of intra-individual stability in the eye blink rate (for example there was a highly significant relationship between the pre-drug eye blink rates on the two separate test days $r = 0.86$ $n = 25$ $p < 0.001$).

There was no significant difference between the pre-drug eye blink rates of the acute schizophrenics and those of the chronic schizophrenics. There was no significant effect of APO on eye blink rates in either group - in both groups there was a slight increase in eye blink rates half an hour after APO administration but this was matched by a comparable increase in eye blink rates after placebo.

C) Correlation between hormonal and clinical ratings

A whole series of correlations between all of the measured variables was performed. Very few significant relationships were established. These are outlined below for the whole group

studied together with several cases where a lack of correlation is of some importance.

1) Age. There were significant negative relationships between age and a) basal PRL level ($r = -0.43$, $n = 40$, $p < 0.01$) b) reduction in PRL post-APO (whether measured by percentage reduction in PRL compared to baseline ($r = -0.41$, $n = 40$, $p < 0.01$) or finite reduction in PRL in mIU/l ($r = -0.37$, $n = 40$, $p < 0.05$)) and c) the GH increment following APO ($r = -0.31$, $n = 40$, $p < 0.05$). Since there was a close relationship between basal PRL levels and the subsequent APO-induced PRL suppression ($r = 0.59$, $p < 0.001$ for percentage and $r = 0.91$, $p < 0.001$ for finite reduction) it is likely that the effects of age of APO induced PRL suppression are mediated by effect on baseline PRL levels. There was a positive correlation between age and oestradiol levels ($r = 0.48$, $n = 30$, $p < 0.01$). This relationship was also found though non-significantly in the study reported in Chapter 4.

2) GH Increments after APO. There was no relationship between the increase in GH secretion after APO and the suppression in PRL found after APO. There was also no relationship between GH increments and oestradiol levels.

Looking at the patient group alone, it was found that there was a significant relationship between positive symptom ratings and GH increment ($r = 0.36$, $n = 30$, $p < 0.05$ Spearman's Rank Correlation) and a significant negative relationship between negative symptom ratings and GH increments ($r = -0.44$, $n = 30$, $p < 0.05$ Spearman's Rank Correlation). However it was

found that the former relationship was based on the effects of age on both variables. While GH increments were found to fall with increasing age across the schizophrenic groups, the frequency of positive symptoms also fell with increasing age. Analysis of these correlations by the method of partial co-efficients (see Appendix E) reduced the relationship between positive symptoms and GH increments to non-significant levels. The relationship between negative symptoms and GH increments however remained significant following this analysis ($r = -.40$, $n = 30$, $p < 0.05$). Moreover this negative relationship was also seen within the chronic patient group alone ($r = -.56$, $n = 15$, $p < 0.05$) whereas there was no evidence of a relationship between positive symptoms and GH increments in either patient group alone.

3) PRL levels. A significant negative relationship was established between basal PRL levels and APO induced PRL suppression and positive symptom scores in the acute schizophrenic group ($r = -0.56$, $n = 15$, $p < 0.05$) but not in the chronic schizophrenic group or in the total schizophrenic group.

4) Eye blink rates. There was no correlation between eye blink rates before or after APO (nor the percentage change between them) and any other variable measured.

DISCUSSION

In summary this study reveals that the only clearcut difference in the hormonal response to a dopamine agonist between acute and chronic schizophrenics and controls was a blunting of the GH response in the chronic schizophrenic group. The effects of apomorphine (APO) administration were similar between the

groups. There was no therapeutic effect of APO on typically schizophrenic symptoms, but a reduction in anxiety was noted in acute schizophrenics following APO administration. Eye blink rates were unaffected by APO and unrelated to clinical state.

The hormonal effects of APO in normal control subjects found here parallel those previously described. There was a large variable increment in GH levels which was maximal at 45 minutes. The variability of GH levels after APO was striking even though most of the known factors which relate to this response were controlled for (i.e. only male subjects were studied and all subjects were fasting and recumbent throughout the procedure). It was found that oestradiol levels were not related to the GH increment in contrast to the study of Ettigi et al (1975) who found large differences in the GH response to APO between males and females and between females on and off oestrogen supplementation. It is probable that oestrogens do suppress this response (and there is animal evidence in support of this notion) but that the effect of oestrogens cannot be seen in the present experiment due to the limited range of oestradiol levels found amongest males. Age was shown to have a significant negative relationship on the GH response to APO in the present experiment. This effect has not been previously described, but no previous study has examined such an age range of subjects. There is however a significant methodological problem in the interpretation of this age effect on GH secretion since it was found across a mixed group of acute and chronic schizophrenics and controls and therefore may be brought about by the effects of illness, rather

than age.

PRL suppression following APO administration leads to similar interpretative difficulties. As was mentioned in the theoretical section in chapter 2 (section 1.3) and again in the results section of the present chapter, there are several ways in which this suppression can be assessed. Since PRL levels fall to a certain extent during the placebo administration, the best method of assessment is to take this into account and calculate the specific (APO - placebo) APO induced suppression. Using this method, the mean specific APO-induced PRL reduction was 20% in the controls. It was apparent that there was a strong effect of the baseline PRL level on the specific reduction associated with APO (i.e. the higher the PRL level the greater the reduction induced by APO). This is in agreement with previous reports (e.g. Rotrosen et al, 1978) and fits in with the observations from dose-response studies where it has been shown that there is a PRL level below which even massive doses of APO do not further suppress PRL levels.

LH levels were unaffected by APO administration in any group studied. This supports previous observations. This lack of effect of APO contrasts with the LH lowering effect of DA infusions. It is probable that the main reason for this contradiction is pharmacodynamic : APO has only a very short half life whereas LH reduction only follows prolonged DA infusion (Kaptein et al, 1980; Huseman et al, 1980). It is possible that the difference lies in the mode or site of action of these two drugs. The evidence is in favour of both drugs exerting

their PRL lowering actions via DA receptors on the pituitary and GH elevation action via the hypothalamus.

Turning to the hormonal results in acute schizophrenics, no clear evidence of any abnormality or change in receptor sensitivity emerged. GH increments after APO showed an enormous variation in the acute schizophrenics, but this variability was not statistically different from that of controls. The cause of this large variability is not clear in controls, far less in acute schizophrenics (see discussion on controls above). This large variation in the GH response to APO in acute schizophrenia has been reported in previous studies (Pandey et al, 1977; Rotrosen, 1979). The time of withdrawal from drugs in these previous studies was short and variable and this lead these authors and others to consider the possibility that neuroleptic withdrawal had induced pituitary receptor supersensitivity in some of the schizophrenic patients leading to a variable response in the group as a whole. However since all the patients in the present study had been off drugs for at least 6 months, and variability was still pronounced, drug withdrawal effects seem an unlikely cause of these widely differing responses.

Another point to consider is that the GH increment after APO is related to clinical state, i.e. that large GH increments are found in a particular subgroup of patients or are related to a particular group of symptoms. No such clinical correlate emerged in the analysis of the results in the present study. No relationship was found between GH secretion and clinical symptoms (such as positive and negative symptoms (or their components)) in

the acute patient group and the group with high GH output were clinically indistinguishable from the low GH output group in terms of age, symptoms, previous episodes etc.

Cleghorn et al (1982) have suggested that clinical state of schizophrenics is not the critical factor which relates to GH secretion after APO. They suggest that GH secretion depends on whether or not this state is changing, i.e. whether the patient is relapsing or remitting. According to this scheme (and the evidence to support it is not overwhelming) patients relapsing or about to relapse have high increments and those in stages of remission have low increments. Unfortunately we have no data from the present study to confirm or refute this hypothesis. It has also been suggested that the magnitude of the GH secretion following APO is positively related to clinical response to drugs (Pandey et al, 1977). This claim has subsequently been retracted (Rotrosen et al, 1979).

Turning to PRL suppression following APO in the acute schizophrenics there was no significant differences between groups by any of the measures used to assess this. The mean specific APO induced reduction (finite reduction in IU/l post APO - finite reduction in IU/l post placebo / basal PRL X 100%) was 16% in the acute schizophrenics (and 20% in the controls). PRL levels were not significantly different between acute schizophrenics and controls. These results are in general agreement with previously published studied (Rotrosen et al, 1979 for review).

It is of interest that there was no relationship between

APO induced PRL suppression and PRL induced GH secretion in either the schizophrenics and controls. This has been reported previously (Rotrosen et al, 1979). This seems to imply that these two hormonal responses are mediated differently. There is experimental evidence to support this contention: APO has a direct effect on suppressing PRL secretion from pituitary cells in vitro but has no effect on GH secretion under these circumstances. Several other lines of evidence indicate that APO induced GH secretion is mediated at the hypothalamic level. For example Brown et al (1982) have demonstrated the domperidone, a peripheral DA blocker, eliminates the PRL response to APO but not the GH response. This implies that the dopamine receptors controlling PRL release are peripheral (located within the pituitary and/or median eminence) while those involved with GH are within the blood brain barrier (perhaps in the anterior hypothalamus where DA receptors have recently been described (List and Seeman, 1981)).

It was found that there was a significant negative relationship between PRL secretion and APO induced PRL suppression and the positive symptoms score of the acute schizophrenics i.e. the greater the positive symptom score the lower the basal PRL and the smaller the reduction in PRL secretion. Since there is a close relationship between PRL suppression and basal PRL levels, the main important relationship is probably between basal levels and symptoms. This relationship is of great theoretical interest, since DA tonically inhibits PRL secretion, and DA has been implicated in the causation of positive symptoms.

This relationship has previously been reported in a group of unmedicated chronic schizophrenics (Johnstone et al, 1977). However in the larger group of acute schizophrenics studied and reported in Chapter 4 only a negative trend between positive symptoms and PRL secretion was demonstrated. Thus it is apparent that the relationship between positive symptoms of schizophrenia and PRL secretion is a weak one and is not applicable to individual patients. It is not really a surprise that this relationship is so weak since so many factors influence PRL secretion and positive symptoms. Nevertheless this relationship is a phenomenon which could repay further study. For example, it has recently been demonstrated (Kleinman et al, 1982) that there is an inverse relationship between positive symptoms and PRL secretion in those schizophrenics with normal CAT scans and where ventricular dilation is present this relationship is not found. This could be considered as evidence that acute and chronic schizophrenia may be separate overlapping syndromes with separate underlying pathophysiologies (Crow, 1980).

The hormonal results from the chronic schizophrenic patients also revealed no evidence of a clearcut abnormality in PRL levels or sensitivity. The specific suppression of PRL following APO administration was 13% of mean basal level in the case of the chronic schizophrenics. This was smaller than either the acute schizophrenic group (16%) or control (20%). However, as mentioned above, statistical analysis revealed that baseline PRL levels were closely related to the degree of suppression caused by APO. PRL levels were lower in the chronic schizophrenic patients and

so this is the likely cause of the reduced APO suppression in this group. Basal PRL levels are known to be lower in chronic, as opposed to acute, schizophrenics (Meltzer et al, 1974). In this study this reduction was shown to be an age effect. This reduction of basal PRL with increasing age was shown in the large series of controls reported in chapter 4 and has been shown in some but not all previously published reports (see chapter 4 for details).

As can be seen from Fig 7.4 and Table 7.2 GH increments after APO were significantly reduced in the chronic schizophrenics compared with acute schizophrenics and/or controls. Blunting of the GH response to APO in chronic schizophrenics has been demonstrated in some (Pandey et al, 1977; Rotrosen et al, 1979) but not all (Meltzer, 1982) previous studies.

The pathophysiological basis of this blunted response is not clear. Rotrosen et al (1979) suggest that this is due to pathological subsensitivity of pituitary DA receptors in chronic schizophrenia induced by chronic neuroleptic therapy. The evidence cited in favour of this contention is that Ettigi et al (1976) found a relationship between blunted GH responses and duration of neuroleptic therapy and that chronic neuroleptic therapy in rats appears to induce subsensitivity of DA receptors in the pituitary (Friend et al, 1978).

However there are several reasons to doubt the validity of this explanation. Firstly the results of Ettigi et al (1976) may relate not to duration of neuroleptic therapy, but to chronicity of illness. Meltzer et al (1982) have described

such a relationship in a large study. This latter finding may relate to the observation of Jeste (1981) who found that a blunted GH response to APO is more common in those patients who have evidence of enlarged ventricles on CAT scan. Further evidence against neuroleptic drugs as the cause of blunted GH response in chronic schizophrenics was found in the present study. Of 5 patients who had never been treated with neuroleptic drugs 3 had GH responses well below the control mean.

There are two further problems with the argument that neuroleptic therapy is implicated in the causation of blunted GH responses in chronic schizophrenia. Firstly, although Friend et al detected biochemical evidence of neuroleptic induced subsensitivity, there is in fact evidence in rats (Lal et al, 1977) of functional supersensitivity to dopamine agonists following neuroleptic withdrawal. Comparable evidence is not yet available in man, but the small study of Brambilla et al (1979) did suggest enhanced GH responses to L-DOPA in the period following neuroleptic withdrawal.

Finally, and most importantly, it is unlikely that pathology of the pituitary dopamine receptor is involved in the mediation of this abnormality, since most evidence (Brown et al, 1978; Brown et al, 1982) points to a hypothalamic site of action for APO in the induction of GH secretion. The exact mechanism of action of APO in the CNS which leads to a GH surge from the pituitary is not clear. APO is virtually certain not to have its GH elevating effect via central DA pathways (see Brown et al, 1978 for review). This effect appears to be mediated either via DA

pathways within the hypothalamus (and associated receptors) or by an action on the hypothalamic releasing hormones involved in GH regulation (GRF and SRIF see chapter 2, section 1.3)

In summary the cause of blunted GH secretion after APO in chronic schizophrenia, which has now been reported by several groups, is not certain. Drugs appear an unlikely cause and in view of the relationships between blunted responses and length of illness, ventricular enlargement and negative symptoms it is conceivable that a degenerative or destructive process in the hypothalamus or closely related structure underlies these impairments.

The significance of this finding is open to doubt until further research can answer some of the questions raised above. A post-mortem examination of pituitary DA receptors from schizophrenics is underway which should throw some light on some of these issues. A further problem is how these findings in a selected (if only by their drug free status) group of chronic schizophrenics relate to the deficits and impairments of chronic schizophrenia population as a whole. This question will be addressed in the next (final) chapter which discusses these issues with respect to all the studies reported here.

The study of the clinical response to APO in schizophrenics produced almost entirely negative results. Nevertheless these results are of interest as they refute some earlier studies which had made some controversial and theoretically important conclusions.

Firstly, it was found that the clinical effects of APO

administration such as sedation and nausea were of comparable frequency and severity in the schizophrenic groups and in the controls. This finding is in direct contrast to that of Meltzer's group (Meltzer, 1980) who reported reduced degrees of sedation to APO (also at a dose of 0.75 mg s.c.) in a group of schizophrenics. On this basis, Meltzer proposed that DA receptors in schizophrenia were subsensitive and that this could lead to a reflex increase in DA metabolism. This hypothesis is not supported by biochemical evidence from post-mortem brain work (see Table 1.1 for details). The experimental data on which this hypothesis rests appears, on the basis of the present results, to be somewhat tenuous. A previous study (Corsini et al, 1977) reported similar results to the present study - sedation, sleep and nausea were "in comparable proportions" following 1 mg of APO in schizophrenics and in normal controls.

There is evidence that the sedative and emetic effects of APO can be blocked by DA receptor blockers and are therefore mediated by DA receptors. Therefore the present study is evidence that there is no alteration in the sensitivity of these receptors in schizophrenia. The exact site and nature of these receptors is not fully established. Nausea/vomiting is probably mediated via DA receptors in the area postrema of the medulla and the effect of APO here is probably on post synaptic receptors. The site of the receptors mediating sedation is unknown. It has been suggested that sedation following APO is mediated by low dose stimulation of pre-synaptic receptors ("autoreceptors") (Meltzer, 1980) but the evidence for this, in man, is not

overwhelming. If autoreceptors are involved in APO-induced sedation, then the dose administered here may have been too high for their preferential stimulation. A study of the clinical effects of low and high dose APO in schizophrenia is probably required to be absolutely certain that there was no change in receptor sensitivity (as far as these responses are concerned) in schizophrenia.

There were no consistent or significant effects of APO administration on the typically schizophrenic symptoms in patients with that diagnosis. Positive symptoms (delusions, hallucinations, thought disorder, incoherence of affect) were not changed after APO administration in either group. Negative symptoms (poverty of speech, flattening of affect) which were found almost exclusively in the chronic schizophrenic group were similarly unchanged. The only significant effect noted was a reduction in anxiety ratings in acute schizophrenics after APO. This appeared to be a specific effect since no change was noted following placebo administration. Anxiety was rated as being present to a morbid degree (score of 2 or more on the Krawiecka scale) in 8 of the 15 acute schizophrenics; 7 of these exhibited a reduction in anxiety, into the non-morbid range (score of < 2). There was no relationship between sedation induced by APO and this reduction in anxiety.

The reduction in anxiety following APO administration in acute schizophrenics is in agreement with the work of Feldman et al (1945) who noted that APO (in a dose of 1.5 mg) was effective in calming excited or agitated psychiatric patients.

These results however do not agree with those of Corsini et al, (1977) and de Chiara et al, (1978) who found that there was a rapid reduction in psychotic symptoms (e.g. delusions, "bizarre behaviour" and "suspiciousness") after APO administration. Both of these studies were effectively open studies and ratings were therefore not performed blind. A 'double blind' study of the clinical effects of APO carried out by Tamminga et al (1978) showed a significant reduction in the New Haven schizophrenia scale (NHSS) score of 18 schizophrenics following 3 mg of APO s.c., but not following identical placebo. The reduction in the NHSS was principally due to a reduction in delusions and hallucinations. The patients were also on neuroleptic medication at the time of study which accounts for the reported tolerance of such a high dose of APO. This, however, leads to interpretative difficulties because an effect of APO on pre-synaptic DA receptors which these authors suggest is the mechanism of the antipsychotic effect would be blocked by concommitant neuroleptic drug exposure. It is conceivable that in schizophrenic brain there could be differential effects of neuroleptics and/or APO on presynaptic receptors, but there is no direct evidence for this notion at present.

The lack of significant effect on positive symptoms is in agreement with that reported by Meltzer's group (Meltzer, 1980) and by Angrist (Angrist et al, 1980 and personal communication). Indeed Corsini has now extended his original study and has found that the antipsychotic effect of APO is restricted to a group of schizo-affective patients (often a group of patients

who are difficult to classify and define). Several groups of workers have reported that ergot derivatives are without antipsychotic efficacy (e.g. Tamminga et al, 1979). The proponents of the theory that APO is antipsychotic suggest that in these studies the dose of ergots may have been too high to achieve selective stimulation of autoreceptors. Further studies are indicated so that these matters can be resolved.

It would appear, taking all the above studies and the present one into account, that the administration of APO does not exacerbate or provoke schizophrenic symptomatology. Because of uncertainty about the receptor effects of the dose range employed in these studies (0.5 - 1.5 mg), a statement about the significance of this finding vis a vis the DA overactivity theory of schizophrenia cannot reliably be made. It could be that presynaptic and postsynaptic effects cancelled themselves out in the present study leading to no change in symptomatology. In partial support of the notion that dose effects are critical, it has been reported that high dose oral APO may have psychotogenic effects in Parkinson's disease. However Angrist et al (1980) reported that APO in doses up to 0.06 mg/kg (~ 4 mg) did not exacerbate schizophrenic symptoms. It could also be postulated that there is no change in receptor sensitivity in schizophrenia or that DA receptors are not involved in schizophrenia. Since there is no evidence for and considerable evidence against these notions (see Table 1.1) these postulates can be discarded for the moment but should be borne in mind.

The results of measuring eye blinks in the schizophrenic patients were almost entirely negative. Since the eye blink rates were not measured in control subjects we cannot directly confirm or deny the observation of Stevens (1978) that blink rates are increased in schizophrenia. The blink rates measured in the schizophrenic subjects accord well with previously published blink rates in normal subjects (e.g. Karson et al, 1982) and are lower than those measured in the schizophrenics studied by Stevens (1978) and Karson et al (1982). The reasons for the difference are not clear : one possibility is the short period of neuroleptic withdrawal in the studies cited above induced a degree of DA receptor sensitivity.

The mean percentage increment in eye blink rates in the schizophrenics after APO was 10%. However the increase in the rate following placebo was 12% which means that no pharmacological effect of APO was demonstrated. An effect of APO on eye blinks has been previously demonstrated only in monkeys (Karson et al, 1982). This discrepancy may represent a true species difference or a dose-dependant difference.

No relationship between eye blink rates and schizophrenic symptoms (positive or negative symptoms or their individual components) was demonstrated in the acute or chronic schizophrenic patients or both groups combined. This confirms the finding of Karson et al (1982). These authors only found a relationship between the change in eye blinks after haloperidol therapy and the change in some aspects of positive symptom ratings (e.g. thought disturbance) after haloperidol therapy. They found this

effect to be more marked in those patients with normal ventricular size. Unfortunately CAT scans were performed only in a small number of the schizophrenic studied here so we cannot comment on the validity of this distinction.

There was no relationship between eye blink rates before or after APO (and the percentage change between them) and APO-induced GH secretion or PRL suppression. Since these latter two responses are mediated by dopaminergic influences, this could be taken as evidence for non-involvement of DA in the blink reflex. However since the anatomical and physiological basis of these responses differ so widely and since these measures are liable to be affected by so many extraneous factors, a statistical comparison (like a correlation co-efficient) is not a valid assessment of the involvement or non-involvement of DA in eye blink rates.

SUMMARY

The results of this study indicate that the administration of apomorphine to schizophrenic patients is not associated with any specific clinical change other than a reduction in anxiety in acute schizophrenics. Blink rates changes and the frequency of 'side-effects' of apomorphine were equally distributed amongst schizophrenics and controls. There was no difference in the PRL suppression induced by APO between the groups but the GH response characteristics of acute and chronic schizophrenics were quite different. The reduced GH secretion of chronic schizophrenics may reflect an abnormality in DA-peptide interaction in long standing schizophrenia.

CHAPTER 8

Concluding remarks: Analysis and synthesis

8.1 General points

The search for the cause of schizophrenia has mirrored social and scientific change. In earlier times demonical possession, witchcraft and vapours were seen as undeniably the cause of madness. In the 19th century descriptive science came to the fore and scientists began to observe and classify psychiatric illness more closely. In the earlier part of the 20th century behavioural and social science held sway and schizophrenia was seen in this light. Since the mid part of the century great strides in physiology, pharmacology and pathophysiology have been made and schizophrenia is now examined in the context of a great wealth of neuroscientific knowledge. Much has been achieved in the management of schizophrenic patients particularly with the advent of neuroleptic drugs and changes in social awareness. However the basic pathophysiology of the disease remains obscure. Part of the problem with research into schizophrenia is that study of the central nervous system is involved. It is clear that the brain has many complex overlapping and interacting anatomical and physiological systems. Another problem is that schizophrenia appears to be a heterogenous disease in terms of symptoms, onset, course and outcome. Consideration therefore should be given to the notion that the clinical condition called schizophrenia represents the end point of various pathophysiological processes in the same way as anaemia can be caused by blood loss or B12 deficiency.

The work presented here is largely concerned with studying schizophrenic patients by means of neuroendocrine techniques. The advantages of this method are that it is relatively non-invasive and can be performed on a variety of patients at different stages of the disease. The principal aims are that either a diagnostic or prognostic marker will be discovered or that a particular pattern of hormonal secretion will be discovered whose elucidation will lead to an understanding of the disease process. As regards the first aim no clear abnormality in schizophrenia or depression has emerged despite considerable research. The DST test in depressive illness has some potential but there are major difficulties evident as outlined in chapter 2 (section 2.2). Research towards the second aim is similarly problematical at present. This leads to a consideration of the major disadvantages of this technique which are discussed below.

The disadvantages of the neuroendocrine approach to studying psychiatric illnesses are basically twofold. Firstly, as was discussed in chapter 2 there are many factors which affect pituitary hormone secretion. Examples include sleep, "stress", age, puberty, glucose and protein intake, posture and drug ingestion. Even if all these factors are controlled for then there is the problem that many of the hormones have a pulsatile secretion. Moreover, when these factors are taken into account the problem of the large individual variation in pituitary hormone secretion remains and leads to interpretative difficulties. Even when an abnormality in pituitary hormone secretion is

reliably identified there is still a considerable problem in ascribing this defect to one neurotransmitter system since it is apparent that several known neurotransmitters have modulating effects on pituitary hormone secretion. A second problem of the neuroendocrine strategy of studying psychiatric patients is that even if a defect of hormonal secretion is identified and the pathophysiological mechanism in terms of neurotransmitters and hypothalamic hormones is established one is still left with the problem that this process may not be representative of events in the remainder of the brain and may be a secondary process.

The disadvantages of the neuroendocrine strategy are however diminished by several other factors. Firstly all other approaches of studying psychiatric patients from the biological point of view have somewhat similar problems. Pharmacological studies are indirect and many of the drugs used lack specificity. Post mortem studies are not suitable for studying dynamic systems e.g. does the findings of (say) an increase in the level of a neurotransmitter reflect increased or decreased secretion and/or turnover? In addition there are some problems with agonal status. Peripheral (urine, blood, CSF) measures of neurotransmitters or their metabolites have not demonstrated consistent changes in psychiatric disease probably partly due to their indirectness. Secondly, in many respects the hypothalamo-pituitary axis responds in a pharmacologically similar way to the rest of the central nervous system and therefore this system is useful for monitoring the central effects of drugs. Finally there is evidence for important hormonal perturbations in both of the major psychoses

(schizophrenia and affective illness), some evidence of disturbed hypothalamic function in these diseases (particularly depression) and possible associations of hormonal disease and both these illnesses. These facts indicate that some disturbance of hypothalamo-pituitary control is likely to be present in both these diseases.

A frequent objection to the studies of the type reported here is that the patients selected for study are unusual and untypical of the total population of schizophrenic patients. A complementary argument is that any differences demonstrated are a result of hospitalisation as an ideal control group cannot be found. In respect of the chronic schizophrenic patients it is true that due to the selection criteria adopted the patients studied were from the unfavourable end of the prognostic spectrum. In fact most of the patients studied had been hospitalised for several years. While a few of the patients had never had neuroleptics, the remainder of the patients' drugs had been stopped due to non-response, non-compliance or both. This also raises the possibility of a selected group of patients. However it was apparent from a study of the casenotes that the patients history and clinical course were very typical of schizophrenia in general. In addition the sample of patient studied here were representative of the total schizophrenic population of their hospital (Owens and Johnstone, 1980) in terms of symptomatology, cognitive functioning and behavioural performance. Finally a similar range and frequency of disabilities have been detected in non-institutionalised outpatient chronic schizophrenics

(Johnstone et al, 1981).

There is no simple answer to the question that prolonged hospitalisation may have been responsible for the neuroendocrine abnormalities reported here although the mechanism of such an effect is far from clear. Research into the disabilities of chronic schizophrenia is always problematical because of the problems of selection of a suitable control group. However there are several pointers against the hypothesis that hospitalisation is involved in the causation of neuroendocrine abnormalities in chronic schizophrenia. Firstly the effects of such an environment have not been noted in humans. In animals reduction in gonadotrophin secretion have been reported in various environmental situations e.g. overcrowding (Taché et al, 1979), exposure to aggressive males (Barkley, 1980) and low position in a hierarchy (Ebehart and Keverne, 1979). The only comparable evidence for an effect on environment on hormones in man is the report of Rose et al (1968) of reduced testosterone in male army personnel under stressful circumstances. Secondly the control group studied here contained some patients which to a certain extent control for the effects of environment. Three long stay patients of the same hospital as the chronic schizophrenic patients were studied. These patients had been hsopitalised for comparable periods with a diagnosis of manic-depressive illness. All of them showed a perfectly normal hormonal profile. A second group of controls for the chronic schizophrenic patients had a history of reactive depression and/or neurotic illness and had been treated in the past with psychotropic medication. This group, to

a certain extent, act as controls for the non-specific effects of psychiatric illness. In the absence of an ideal control group (presumably normal people receiving inpatient care for more than 20 years in a psychiatric hospital) the present mixture of chronically hospitalised non-schizophrenics, depressed or neurotic outpatients and normal controls (not selected on the basis of perfect physical health) seems the best available compromise.

As regards the acute schizophrenic patients studied here there can be little doubt about the diagnosis. All of the patients had a recent onset of positive, florid symptoms of schizophrenia and all had Schneiderian first rank symptoms and fell into the nuclear schizophrenia (NS+) category on Present State Examination. This group of patients is one on which there is most agreement about the diagnosis of schizophrenia.

8.2 Overview of results in context of psychoneuroendocrinology

a) Chronic schizophrenia

In summary the results of these studies demonstrate selective reductions in gonadotrophin secretion in a subgroup of chronic schizophrenics which is associated with a reduction in the frequency but not amplitude of LH secretory episodes. While this pattern of abnormal secretion appeared to be stable and reproducible within individual patients, the administration of acute or chronic DA antagonists was associated with a return towards normal levels. This suggests that DA overactivity may be implicated, at least in part, with the genesis of these abnormalities and this contention is supported by the finding

that those patients with the most marked derangement of gonadotrophin secretion had the lowest prolactin levels. These patients also exhibited the most evidence of hypothalamo-pituitary derangement in that their responses to hypothalamic releasing hormones was disturbed with blunted FSH and PRL responses and aberrant GH responses to TRH/LHRH administration. The GH response to a dopamine agonist was also reduced in chronic schizophrenics as a group and markedly so in a subgroup of the patients, particularly those with predominant negative symptoms.

The clinical associations of these abnormalities have not yet been fully established. This is partly due to the relatively small numbers of patients studied but also to the general difficulty of comparing a biological variable with a clinical or phenomenological one. For example it is more meaningful to look for correlations across an entire group of patients or should one divide them into groups on one or other vairable and then do comparisons? Both methods have the inherent danger that the greater number of relationships examined the greater number liable to be significant by chance.

With these reservations in mind it appears from these and other studies that in chronic schizophrenia:-

a) FSH secretion is lowest in those patients with the most positive symptoms and the earliest age of onset (Shader et al, 1969; Johnstone et al, 1978 and present study).

b) LH secretory profiles are most deranged in those patients with the longest length of illness and there is a trend for more marked abnormalities in patients with more prominent positive symptoms.

c) PRL secretion does not relate to psychopathology in chronic deteriorated patients (this study and Kleinman et al, 1982) and

d) GH secretion to apomorphine is reduced in those patients with the longest history , those with CAT scan deficits (Meltzer et al, 1980; Jeste, 1981)and negative symptoms (see below).

The most likely explanation of these hormonal findings and clinical associations is that in chronic schizophrenia there are abnormalities of DA - hypothalamic peptide interaction. The reduced LH and FSH secretion are probably related to reduced LHRH secretion from the hypothalamus. The evidence for this was discussed in chapters 4 and 5. DA blockade to a certain extent reversed these abnormalities. DA has been shown to play a role in the extracellular degradation of LHRH in the hypothalamus (Marcanno de Cotte, 1980). There is a close anatomical relationship between DA and LHRH in the median eminence (McNeil and Sladek, 1978). It is therefore conceivable that in schizophrenia excess DA inhibits or reduces LHRH release and this effect is reversed when DA blockade is induced. The site of action of dopamine blockers in the mediation of this response is however not clear. It would be of interest to observe if domperidone (a DA blocker which does not cross the blood - brain barrier) also induced a rise in gonadotrophin secretion in chronic schizophrenia. The association of reduced FSH secretion and increased positive symptoms and the similar trend for LH secretion is of interest since DA blockade tends to ameliorate both reduced gonadotrophin secretion and positive symptoms. This

would seem to support the contention that DA overactivity is a process underlying the positive symptoms of schizophrenia (Crow, 1980).

As was discussed at length in chapter 7 it is probable that the GH elevating effects of apomorphine are mediated within the hypothalamus. It is probable that such an effect is mediated by either a release of the putative hypothalamic peptide GH releasing hormone (GHRH) or an inhibition of somatostatin (GH inhibiting hormone or SRIF) from the hypothalamus. The blunting of the GH response to APO could therefore result from a deficiency of GHRH release, an excess of SRIF release or a defect of the apomorphine-peptide interaction. The further elucidation of this deficit must await more detailed knowledge of the mechanism of APO induced GH secretion.

Blunting of GH response to APO has been found to relate to increasing length of illness (Meltzer et al, 1980) and to CAT scan abnormalities (Jeste, 1981) in schizophrenics. In this study the patients with blunted responses tended to be those with long histories, those with negative symptoms (possibly a marker of organic deterioration of schizophrenic patients (Owens and Johnstone, 1980)) and those with poor cognitive performance. The relationship with symptoms reached statistical significance. What is clear is that the abnormal blunted response does not relate to positive symptoms in the same way as gonadotrophin secretion appears to. The clinical associations tend to point towards this blunting related to some organic, perhaps destructive process, in the hypothalamus. The clinical

associations of the two basic hormonal deficits (reductions in LH and FSH secretion and blunted GH responses to APO) may indicate separate processes underlying them supporting Crow's (1980) hypothesis (see p 22(

It is difficult to conceive of one process that could lead to these somewhat selective deficits and leave the remainder of the HPA axis working normally. A lesion in the supra-charismatic nucleus could reproduce the LH secretory pattern seen in these patients but is unlikely to be associated with GH response blunting. However it is possible that the anterior hypothalamus in which both LHRH pathways (Barry, 1977) and dopamine receptors (List and Seeman, 1981) have recently been observed could be an important area to investigate in schizophrenic brain. It is also conceivable that one neurotransmitter abnormality underlies both these abnormalities and the symptoms of schizophrenia. A long lasting abnormality may lead to adaptive changes in biochemical and receptor function that may subsequently be difficult to separate.

The question of long lasting changes in neurotransmitter function and secondary adaptive change is relevant to the discussion of the PRL stimulation test results of the chronic schizophrenics. The PRL response to TRH was blunted and aberrant GH responses were seen in some cases. On the other hand the PRL responses to both DA antagonist (metaclopramide) and agonist (apomorphine) were normal. This pattern of response is distinct from that seen in cases of known organic lesions of the hypothalamus (Ferrari et al, 1982). In these cases the PRL response to TRH is more or less preserved but that to DA blockade is

abolished. However it is probable that the chronic hyperprolactinaemia present in these cases is responsible for this pattern: it appears to induce impaired DA levels within the pituitary itself possibly by a short-loop feedback on DA turnover. The results from schizophrenic patients indicate that DA receptors, DA levels and PRL reserve of the pituitary are normal. The blunted TRH response indicates either an abnormality of the TRH receptor on the lactotrophe itself or some change in dopaminergic tone within the HPA. There is however some dispute on the effect of DA tone on the PRL response to TRH: Healy and Burger (1977) reporting opposite effects from Kauppila et al (1982). Paired testing with TRH and DA blockers in the same patients is recommended to separate these various possibilities (Ferrari et al, 1982).

Two final problems need to be discussed before leaving the chronic schizophrenic studies. These are 1) what is the significance of the fact that these changes are only found in a subgroup of patients and 2) are the defects specific for diagnosis. Some of these matters have been dealt with in the discussion sections of chapters 4, 5, 6 and 7 but an overview is presented here.

In general terms consistent abnormalities of pituitary hormone secretion were only detected in about 50% of the chronic schizophrenics studies. 25% were in the normal range on every variable tested and the remainder were in the equivocal range. The cause of this heterogenity is not at all clear at present. A similar situation is encountered in neuroendocrine studies

with depressed patients. This elucidation of this heterogenity represent a major challenge to psychiatrists and neuroendocrinologists. Mis-diagnosis is unlikely as all of the patients studied appeared to have had classical schizophrenic illness. It is conceivable that the endocrine differences within chronic schizophrenics reflect different underlying disease processes but there is no confirmatory evidence of this at present.

The specificity of these changes to chronic schizophrenics is somewhat uncertain. However it does appear that the particular pattern of changes is different from several other important psychiatric, neuropsychiatric and endocrinological conditions as outlined below.

1) Affective illness. While there are unconfirmed reports of reduced LH, FSH and LH cycling in depression (see chapter 2, section 2.2), there are several dissimilarities between the results in chronic schizophrenics and depression. For example the TSH response to TRH is blunted in a number of depressives but is normal in schizophrenia. The GH response to apomorphine is normal in depression but abnormal in chronic schizophrenics. Differences found in manic or bipolar affective patients were outlined in chapter 2(section 2.2).

2) Anorexia nervosa. While there are some similarities in gonadotrophin secretion between anorexia nervosa and chronic schizophrenia the most important differences are that in schizophrenia abnormalities do not relate to body weight and that testing with LHRH and/or TRH produces a completely

different pattern of results.

3) Dementia. No clear abnormalities of pituitary hormone secretion have been identified in dementia.

4) Neurotic illness. Stress in females has been reported to impair the LH secretory profile. Such changes are often associated with low PRL levels. The PRL responses to TRH and GH response to DA agonists is usually within normal limits in these cases.

Similarities and differences between the hormonal profile of chronic schizophrenic patients and I.G.D. patients and organic hypothalamic conditions have been outlined in chapter 5 and above.

Thus the abnormalities described here exhibit diagnostic specificity for the diagnosis of schizophrenia, but only when the entire range of abnormalities is considered. The relationship of these abnormalities to subgroups of patients with reported evidence of testicular pathology, reduced fertility, menstrual abnormalities and reduced urinary testosterone (discussed in chapter 1) remains to be established.

b) Acute schizophrenia

No clear-cut abnormalities of pituitary hormone secretion were detected in this group of patients. Some potentially interesting results relating hormone secretion to clinical symptoms, drug dosage and levels were demonstrated.

The levels of several hormones notably LH, FSH, GH and PRL

were unaltered compared to an age-matched control group. The normality of LH and FSH secretion, the lack of any change in them following neuroleptic medication and the evidence of normal episodic LH secretion demonstrated in this group of patients compared to the chronic patients is of interest since it indicates that gonadotrophin secretion abnormalities are unlikely to be aetiologically significant in schizophrenia as was postulated by amongest others Kraepelin (1919) and McCartney (1929). Rather these abnormalities may be an index of processes involved with chronicity.

Basal GH secretion was normal in these patients as was the overall GH response to a DA agonist. There was a very marked variation in this response with some patients with a markedly blunted response and others with an exaggerated response. This elucidation of this pattern (previously reported by others e.g. Rotrosen, 1979) may prove to be a useful clinical indicator particularly as blunting is seen in a very much higher proportion of chronic schizophrenics. One possible cause of this large variation - the effects of neuroleptic drug withdrawal - was excluded by the present study.

PRL secretion was not abnormal in these patients. This could be taken as evidence against the DA hypothesis of schizophrenia as was discussed in chapter 4. However in view of the short-loop feedback control system of PRL secretion it is conceivable that PRL levels tend to return to normal even in the face of a perturbation in the DA system. In support of this thesis is the trend (significant in some groups) for a negative relationship between PRL secretion and positive symptom scores.

Thus those patients with the greater positive symptoms and therefore the best likelihood of a therapeutic response to a DA blocker were those with the lowest PRL levels and by extrapolation possibly the highest hypothalamic DA release. This relationship may become more clinically relevant in the future.

A close relationship between PRL secretion and neuroleptic dose and blood levels was established but PRL secretion did not relate to clinical improvement nor to drug induced side effects. The main problem with these types of relationship is that they can only be tested in groups of patients : there are too many exceptions in the individual case to make these observations clinically relevant.

3. Results other than neuroendocrine

The important non-neuroendocrine findings of these studies were that apomorphine administration is not associated with any clinical improvement in schizophrenics and that the frequency of side effects was similar in controls and schizophrenics. This study, carried out double-blind on unmedicated patients, weakens the experimental basis of a number of hypotheses which have been recently put forward. Such studies are not easy to perform but as discussed in chapter 7 there remains a need to explore the clinical effects of different doses of dopamine agonists in schizophrenia.

No effects of apomorphine on blink rates were demonstrated. This puts doubt on the relevance of the changes in blink rates in schizophrenics on and off medication. Recently Karson has found no effect of L-DOPA on human blink rates (personal

communication) which concurs with the results of the present study. Clearly the pathophysiological significance of blink rates to psychiatry requires much further evaluation.

8.4 Future work

Much further experimentation is required in the field of neuroendocrinology as applied to psychiatric disease. At present there are too many variables and inconsistencies to allow proper evaluation of true deficits. Possible lines of enquiry in schizophrenia include:-

a) Tests on patients

1) More cross diagnostic studies need to be done.

2) A study of the effect of acute and chronic DA antagonists in a range of acute, subacute and chronic schizophrenic patients on LH and FSH secretion may indicate more clinical associations of this response.

3) The clinical and hormonal effects of repeated small doses of synthetic LHRH on schizophrenic patients requires evaluation.

4) The only other drug which consistently enhances LH episodic secretion in the opiate antagonist naloxone (Moult et al, 1981). This drug was also effective in restoring LH episodic release in patients with hypothalamic amenorrhoea who responded to metaclopramide (Quigley et al, 1980). A combined clinical and endocrinological study with naloxone in schizophrenics may prove interesting.

5) A more complete analysis of gonadal steroids other than total testosterone and oestradiol is indicated in view of the abnormalities detected by Hoskins and Pincus (1949) (see chapter

1) and the observations on the different relationship between LH and age and testosterone secretion in schizophrenics reported in chapter 4.

6) Measurements of LHRH and SRIF in blood and CSF are being developed and may correlate interestingly with the neuroendocrine changes in schizophrenics.

b) Post-mortem studies

1) A more complete analysis than previously performed of pathological states in pituitary, hypothalamus and testes of schizophrenics needs to be performed.

2) Measurements of neurotransmitters, receptors and peptides (e.g. LHRH and SRIF) in accurately dissected areas of the hypothalamus in clinically documented patients are indicated to complement these neuroendocrine investigations of living patients.

APPENDICES

APPENDIX A

Feighner criteria for diagnosis of schizophrenia

These criteria (Feighner er al, 1972) were applied to the casenotes of hospitalised chronic schizophrenics. The chronicity of the illness was further established by adopting a policy of one year's continuous hospitalisation.

Schizophrenics

For a diagnosis of schizophrenia A through C are required

A. Both of the following are necessary:

1) A chronic illness with at least six months of symptoms prior to the index evaluation without return to the premorbid level of psychosocial adjustment.

2) Absence of a period of depressive or manic symptoms sufficient to qualify for affective disorder or probable affective disorder.

B. The patient must have at least one of the following:

1) Delusions or hallucinations without significant perplexity or disorientation associated with them.

2) Verbal production that makes communication difficult because of a lack of logical or understandable organization.

C. At least three of the following manifestations must be present for a diagnosis of "definite" schizophrenia and two for a diagnosis of "probable" schizophrenia.

1) Single.

2) Poor premorbid social adjustment or work history.

3) Family history of schizophrenia.

4) Absence of alcholism or significant drug abuse within one year of onset of psychosis.

5) Onset of illness prior to age 40.

Feighner et al (1972) state that they have demonstrated 87 - 95% inter-rater concordance in the diagnosis of schizophrenia. Of the 28 chronic schizophrenics studied here 20 were classified as "definite" and 8 as "probable" schizophrenia.

28 chronic schizophrenics were studied. 18 of the 20 who took part in the study reported in chapter 4 took part in the study reported in chapter 5. 3 of these patients were restudied in chapter 6 (effects of chronic neuroleptics). The effects of metoclopramide were studied in 10 patients - this number included 5 patients never previously studied and 5 of the 18 discussed above. 15 patients were given apomorphine including the 5 new patients discussed above, 3 additional never studied patients and 7 of the original cohort of 18 (above). No patient took part in more than two studies.

APPENDIX B

The Present State Examination (PSE)

The Syndrome check list of the PSE (Wing et al, 1974) was applied to the casenotes of the chronic schizophrenic population identified by the Feighner criteria. The features of the illness at its worst were examined. The diagnostic categories in which the patients were placed were:- NS (nuclear schizophrenia - 7 patients), DS (schizophrenia without first rank symptoms - 13 patients), DS? (possible schizophrenia without first rank symptoms - 6 patients), CS (catatonic schizophrenia - 1 patient and DP/AP (paranoid psychoses combined with affective psychoses - paranoid psychosis predominant - 1 patient). These categories show a high degree of agreement between the PSE and Feighner criteria for schizophrenia. 'NS' and 'DS' cannot really be separated in this samples, as the fact that nuclear symptoms were not recorded in the casenotes does not mean that they were not present and indeed it is likely that in many cases they were not specifically sought. The DS? category results from insufficent information in the casenotes. The DP/AP category is one of possible schizophrenia. The syndrome check list records not only symptoms but also behaviour. The DP/AP category results from a recording of 'excitement' which comes under the classification of an affective behaviour (but which of course could occur for several reasons including schizophrenia). Thus the PSE classification supported the diagnosis of schizophrenia to a large extent. However it is not primarily a method of diagnosis rather of classifying an identified group

of patients. This classification did not relate to the neuro-endocrine changes as has been mentioned in the main text.

The acute schizophrenics (as defined in chapter 3 section were examined by an interview constructed round the Present State Examination (Wing et al, 1974). A total 48 acute schizophrenics were included in the present studies. All of them were classified as NS (nuclear schizophrenia) on the PSE classification.

Of the 48 acute schizophrenics, 14 were female and were included only in the study on anticholinergics and neuroleptics on clinical state and anterior pituitary secretion reported in chapter 6. 3 patients were acute patients in remission and results from these patients were reported in chapter 4. Of the remaining 31 male acute schizophrenics, 15 were studied in the apomorphine study (chapter 7). The remaining 16 along with 6 of the patients given apomorphine took part in the neuroleptic study (chapter 6).

APPENDIX C

Krawiecka scale for rating chronic psychotic patients

This scale - "standardized psychiatric assessment scale for rating chronic psychotic patients" was published in 1977 by Krawiecka, Goldberg and Vaughan. Videotaped interviews were made of known psychotic patients. A manual of guidelines for the rating of each symptom or sign was produced. There were 4 ratings based on replies to questions (depression, anxiety, delusions and hallucinations) and 4 ratings based on observation (incoherence of speech, poverty of speech, flattened incongruous affect and psychomotor retardation). Each rating was made of a 5 point scale. Examples from both kinds of ratings are given below. In the present study flattening and incongruity of affect were rated separately. Krawiecka et al demonstrated significant co-efficients of concordance for each of the ratings except that related to flattened incongruous affect. This concordance applied to psychiatrists without prior experience of the rating scale.

Example (1) Coherently expressed delusions

Rating 0 "Absent" No abnormality detected at interview.

1 "Mild" Eccentric beliefs and trivial misinterpretations e.g. that bad weather is cuased by nuclear tests, superstitions etc.

2 "Moderate" Over valued ideas and ideas of reference or undoubted misinterpretations. Special meanings.

3 "Marked" Undoubted delusions or delusional perception are described as having occurred in last month,

but the patient denies that he still holds the beliefs or delusional ideas are still held but they are not strongly held or incorrigible.

4 "Severe" Undoubted delusions are present and are still held by the patient.

Example (2) Poverty of speech, mute

Rating 0 "Absent" Speech normal in quantity and form.

1 "Mild" Patient only speaks when spoken to; tends to give brief replies.

2 "Moderate" Occasional difficulties or silences but most of interview proceeds smoothly or conversation impeded by vaguness, hesitancy or brevity of replies.

3 "Marked" Monosyllabic replies; often long pauses or failure to answer or reasonable amount of speech but answers slow and hesitant, lacking in content or repetitions and wandering so that meaningful conversation was impossible.

4 "Severe" Mute throughout interview or speaks less than 3 words.

APPENDIX D

Radioimmunoassay

1) Iodination of proteins

a) LH, FSH and GH were iodinated by the chloramine T (CT) method. CT oxidises iodide to iodine which can then be attached to tyrosine residues on the peptide chain.

Method (1) 25 µl of 0.25M PO_4 was added to 0.5 mCu $Na^{125}I$.

(2) Above mixture was added to 2 µg or 3 µg portions of proteins (see table 3.1 for details).

(3) 15 µl of 3.5 mg/ml CT was added.

(4) After 15 seconds 25 µl of 4.8 mg/ml sodium metabisulphite was added.

(5) 100 µl of human serum albumin was added.

(6) Reaction mixture was added to a G50 column for initial lesaltry for all protein.

(7) Protein fraction peak was added to either a 12 ml G150 column or a 60 cm ACA 44 column and 500 µl fractions were collected and tested.

b) PRL was iodinated by the lactoperoxidase method, an enzyme that induces slower and more gentle oxidation.

Method (1) 30 µl of 0.5M PO4 was added to 0.5 mCu of $Na^{125}I$.

(2) Above mixture was added to 3 µg of HPRL.

(3) 4 µl of lactoperoxidase was added to above.

(4) After 20 mins 2 µl of hydrogen peroxidase (10 µl of 30% w/v in 100 ml) were added.

(5) After a further 10 mins another 2 µl of H_2O_2 was added.

(6) 500 µl of buffer (Table 3.1) was added and the mixture applied to a 60 cm ACA 44 column and 500 µl fractions collected.

The fractions were tested for specific binding by incubating aliquots of the column fractions with and without antiserum at assay concentrations (Table 3.1) and suitable fractions were stored at -40° until assay time. Specific binding was of the order of 40 - 60% for each of these proteins and non-specific binding 2%. Iodinated material could be used for 1 - 2 months.

2) Dose-response curves (D-R curves)

D-R curves were generated by a computer program written by Dr. R. Wooton based on Healey's (1972) log-dose logit-response standard curve. Several concentrations above and below the detection limit of the assay were included so that the ends of the curve were assymptotic using this plot. The slope of the curve was effectively linear in the regions of assay sensitivity (15 - 85 percent of total bound).

3) Quality control data

As was discussed in chapter 3 (section 3.8) it is most important to run quality control samples in each assay. The data for quality control samples for each series of studies for the hormones measured has been given in the appropriate chapter (4, 5, 6, 7). The overall co-efficients of variation over all the assays performed are given below.

	Total number of assays	Co-efficient of variations (%)		
	n	High QC	Medium QC	Low QC
PRL	23	15	8	10
LH	22	15	8	11
FSH	13	10	7	7
GH	16	16	11	13
Testosterone	4	11	-	15
TSH	5	11	-	10
Oestradiol		External quality control at Chelsea Hospital		

It can be seen that co-efficients of variation were lower in the medium range of quality control and the errors were greater (though acceptable) towards the levels of sensitivity. This type of 'precision profile' for an assay is the common experience and is important to examine prior to interpretation of the experimental data.

APPENDIX E

Partial correlation co-efficients

When 3 variables have been measured and one is interested in the relationship between them the following formula is useful:-

$$r12.3 = \frac{r12 - r\,13 \cdot r23}{\sqrt{(1-r13^2)\ (1-r23^2)}} \qquad df = n-3$$

where 1,2 and 3 are the variables

r12 is the correlation between variables 1 and 2

r13 is the correlation between variables 1 and 3

and r23 is the correlation between variables 2 and 3

r12.3 is then the correlation co-efficient between variables 1 and 2 taking their relationship with variable 3 into account. In other words, r12.3 tells one if the relationship between variable 1 and variable 2 is dependant or independant of their relationship with variable 3 - if r12 is significant but r12.3 is not then significance is attributable to relationships with variable 3.

DECLARATION OF WORK DONE BY AUTHOR

The idea for these studies came from discussions involving Dr. T.J. Crow, Dr. E.C. Johnstone and myself but the design of each study and the appropriate search of the literature was my own. Dr. E.C. Johnstone and Dr. D.G.C. Owens identified, classified and rated the schizophrenic patients but I made all the arrangements for these patients to be studied (including discussion with relatives). I identified and classified all the controls studied.

All venous sampling and preparation of sera was performed by the author. I also performed all the radioimmunoassays with the exceptions documented in the text (T_3, T_4 assay - Miss S. Copping, oestradiol - Dr. R. Rodriguez and flupenthixol - Miss R. Bourne). I prepared all the solutions and iodinated material with some technical assistance from Mr. W. Bartlett and Miss C. Canning in the early stages. Clinical ratings and eye blink ratings in the apomorphine study were carried out by Dr. E.C. Johnstone and myself, with some help from Dr. T.J. Crow. Drs. Johnstone and Owens performed the clinical ratings and extra-pyramidal ratings described in chapter 6. Mr. S. Gamble wrote a microcomputer programme which aided eye blink rate measurements and he also provided some assistance with statistical packages. All statistics were performed by myself following discussions with Drs. P. Royston and C.D. Frith.

In writing up these studies I have drawn on papers written by Drs. Crow and Johnstone and many useful ideas have come from discussion with these workers. Nevertheless the ideas expressed

in the introductions and discussions of this thesis are my own and come from my own survey of the literature and review of these results.

Signed: Neil Ferrier Date: 28th January 1983

DR. I.N. FERRIER

REFERENCES

Altman, N., Sachar, E.J., Gruen, G.H., Halpern, F.S. and Eto, S. (1975) Reduced plasma LH concentration in post-menopausal depressed women. Psychosom. Med. 37, 274-276.

Angrist, B., Lee, H.K. and Gershon, S. (1974) The antagonism of amphetamine induced symptomatology by a neuroleptic. Am. J. Psychiat. 131, 817-819.

Angrist, B., Rotrosen, J. and Gershon, S. (1980) Responses to apomorphine, amphetamine and neuroleptics in schizophrenic subjects. Psychopharm. 67, 31-38.

Asano, N. (1967) Pneumoencephalographic study of schizophrenia In Clinical Genetics in Psychiatry. Problems in nosological classification. Ed. Mitsuda, H. and Takasuki, O. Osaka Medical College, Osaka, Japan.

Asnis, G.M., Sachar, E.J., Halbreich, U., Nathan, R.S., Ostrov, L., Solomon, M. and Hapern, F.S. (1981) Endocrine responses to thyrotrophin-releasing hormone in major depressive disorders. Psychiat. Res. 5, 205-15.

Baker, H.W.G., Burger, H.G., de Kreitser, D.M., Hudson, B., O'Conner, S., Wong, C., Merovics, A., Court, J., Dunlop, M. and Rennie, G.C. (1976) Changes in the pituitary-testicular system with age. Clin. Endocrinol. 5, 349-372.

Barkley, M.S. (1980) Concentrations of luteinizing hormone in the serum of male mice after short-term aggressive interaction. J. Endocr. 85, 299-305.

Barry, J. (1977) Immunofluorescence study of LRF neurons in man. Cell Tiss. Res. 181, 1-14.

Belchetz, P.E., Plant, T.M., Nakai, Y., Keogh, E.J. and Knobil, E. (1978) Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotrophin-releasing hormone. Science 202, 631-633.

Ben-Jonathan, N., Oliver, C., Weiner, H.J., Mical, R.S. and Porter, J.C. (1977) Dopamine in hypophysial portal plasma of the rat during the oestrous cycle and throughout pregnancy. Endocrinol. 100, 452-458.

Berson, S.A. and Yallow, R.S. (1968) General principle of radioimmunoassay. Clin. Chem. Acta. 22, 51-69.

Besses, G.S., Burrow, G.N., Spaulding, S.W. and Donabedian, R.K. (1975) Dopamine infusion acutely inhibits the TSH and prolactin responses to TRH. J. Clin. Endocrinol. Metab. 41, 985-988.

Beumont, P.J.V., Corker, C.S., Friesen, H.G., Gelder, M.G., Harris, G.W., Kolakowska, T., Mackinnon, P.C.B., Mandelbrote, B.M., Marshall, J., Murray, M.A.F. and Wiles, D.H. (1974a) The effects of phenothiazines on endocrine function. I. Patients with inappropriate lactation and amenorrhoea II. Effects in men and post-menopausal women. Br. J. Psychiat. 124, 413-430.

Beumont, P.J.V., Freisen, H.G., Gelder, M.G. and Kolakowska, T. (1974b) Plasma prolactin and luteinizing hormone levels in anorexia nervosa. Psychol. Med. 4, 219-221.

Birley, J.L.T. and Brown, G.W. (1970) Crises and life changes

preceding the onset or relapse of acute schizophrenia: clinical aspects. Brit. J. Psychiat. 116, 327-333.

Bjorklund, A., Lindvall, O. and Robin, A. (1975) Evidence of an incerto-hypothalamic dopamine neurone system in the rat. Brain Res. 89, 29-42.

Blair, J.H., Sniffen, R.C., Cranswick, E.H., Jaffe, M.D. and Kline, N.S. (1952) The question of histopathological changes is the testes of the schizophrenic. J. Ment. Sci. 98, 464-465.

Bleuler, E. (1902) Dementia praecox. J. Ment. Path. 111, 113-120.

Bleuler, E.P. (1911) Dementia praecox or the group of schizophrenias (trans Zinkin, J.) International Universities Press, New York.

Bliss, E.L., Migeon, C.J., Branch, C.H.H. and Samuels, L.T. (1955) Adrenocortical function in schizophrenia. Am. J. Psychiat. 112, 358-65.

Bloom, F.E., Segal, D., Ling, N. and Guillemin, R. (1976) Endorphins: profound behavioural effects in rats suggest new etiological factors in mental illness. Science 194, 630-632.

Bonhoeffer, K. (1909) Zur Frage der exogenen Psychosen. Zentbl. Nervenheilk, 32, 499-505 (Translated and reprinted in Themes and Variations in European Psychiatry, edited by S.R. Hirsch and M. Shepherd, John Wright, Bristol, 1974).

Bowers, M.B. (1974) Central dopamine turnover in schizophrenic syndromes. Arch. Gen. Psychiat. 31, 50-54.

Boyar, R., Perlow, W., Hellman, L., Kapen, S. and Weitzman, E. (1972) Twenty-four hour pattern of luteinizing hormone secretion in normal men with sleep stage recording. J. Clin. Endocrin. Metab. 35, 73-81.

Brambilla, F., Guerrini, A., Riggi, F. and Ricciardi, I. (1974) Psychoneuroendocrine investigation in schizophrenia: relationship between pituitary gonadal function and behaviour. Dis. Nerv. Syst. (Aug) 362-367.

Brambilla, F., Guerrini, A., Guastalla, A., Rovere, C. and Riggi, F. (1975) Neuroendocrine effects of haloperidol therapy in chronic schizophrenia. Psychopharmacol. 44, 17-22.

Brambilla, F., Rovere, C., Guastalla, A., Guerrine, A. and Riggi, F. (1976) Gonadotrophin response to synthetic gonadotrophin hormone-releasing hormone (GnRH) in chronic schizophrenia. Acta Psychiat. Scand. 54, 131-145.

Brambilla, F., Rovere, C., Guastalla, A., Guerrine, A., Riggi, A. and Burbate, A. (1977) Effects of clomiphene citrate administration on the hypothalamo-pituitary gonadal axis of male chronic schizophrenics. Acta. Psychiat. Scand. 56, 399-406.

Brambilla, F., Smeraldi, M., Sacchetti, E., Negri, F., Cocchi, F. and Muller, E.E. (1978) Deranged anterior pituitary responsiveness to hypothalamic hormones in depressed patients. Arch. Gen. Psychiat. 35, 1231-1238.

Brambilla, F., Bellocli, L., Negri, F., Smeraldi, E. and Malagoli, G. (1979) Dopamine receptor sensitivity in the hypothalamus

of chronic schizophrenics after haloperidol therapy: growth hormone and prolactin response to stimuli. Psychoneuroendocrinol. 4, 329-339.

Brooksbank, B.W.L. and Pryse-Phillips, W. (1964) Urinary Δ^{16}-androst-16-en-3α-ol, 17-oxosteroids and mental illness. Brit. Med. J. 1, 1602-6.

Brooksbank, B.W.L., MacSweeney, D.A., Johnson, A.L., Cunningham, S.E., Wilson, D.A. and Coppen, A. (1970) Androgen excretion and physique in schizophrenia. Brit. J. Psychiat. 117, 413-420.

Brown, G.M., Seeman, P. and Lee, T. (1976) Dopamine/neuroleptic receptors in basal hypothalamus and pituitary. Endocrinol. 99, 1407-1410.

Brown, G.M., Seggie, J.A., Chambers, J.W. and Ettigi, P.G. (1978) Psychoneuroendocrinology and growth hormone: a review. Psychoneuroendocrinol. 3, 131-153.

Brown, G.M., Verhaegen, H., van Wimersma and Brugmans, J. (1982) Endocrine effects of domperidone: a peripheral blocking agent. Clin. Endocrinol. 15, 275-283.

Brown, W.A., Laughren, T.P. and Robzyk, P.H. (1979) Serum prolactin in humans parallels neuroleptic induced changes in dopamine receptor sensitivity. Society for Neurosciences, 9th Annual Meeting, Atlanta, Georgia, Extract No. 1482.

Bullmore, G.H.L. and Coppen, A. (1965) Effects of chorionic gonadotrophin on adolescent psychiatric patients. Brit. J. Psychiat. 111, 733-739.

Burt, D.R., Creese, I. and Snyder, S.H. (1977) Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196, 326-328.

Byfeld, P.G.H., Bird, D., Land, M., Yepez, R. and Himsworth, R.L. (1979) Inter-relation of the pituitary-thyroid system st birth in man. J. Endocrin. 80, 373-379.

Caraceni, T.A., Parate, E.A., Cocchi, D., Panerai, A.E. and Muller, E.E. (1977) Altered growth hormone and prolactin responses to dopaminergic stimulation in Huntington's Chorea. J. Clin. Endocrinol. Metab. 44, 870-875.

Carroll, B.J., Feinberg, M., Steiner, M., Haskett, R.F., James, N. McI and Tariha, J. (1980) Diagnostic application of the dexamethasone suppression test in depressed outpatients In: Advances in Biological Psychiatry. J. Mendlewicz. Karger.

Checkley, S.A. (1979) Corticosteroid and growth hormone responses to methylam phetamine in depressive illness. Psychol. Med. 9, 107-115.

Checkley, S.A. (1980) Neuroendocrine tests of monoamine function in man; a review of basic theory and the application to the study of depressive illness. Psychol. Med. 10, 35-53.

Cleghorn, J.M., Brown, G.M., Brown, P.J., Kaplan, R.D., Dermer, S.W., McCrummon, D.J. and Mitton, J. (1982) Growth hormone responses to apomorphine HCl in schizophrenic patients (1) Dose response curves (2) Following neuroleptic withdrawal. Arch. Gen. Psychiat. (in press).

Collins, W.P., Mansfield, M.D., Allandina, N.S. and Somerville, I.F.

(1972) Radioimmunoassay of plasma testosterone. J. Steroid. Biochem. 3, 333-348.

Connell, P.H. (1958) Amphetamine psychosis. Maudsley Monograph 5. Oxford University Press, London.

Corsini, G.U., del Zompo, M., Manconi, S., Cianchetti, C., Mangoni, A. and Gessa, G.L. (1977) Sedative, hypnotic and antipsychotic effects of low doses of apomorphine in man. Adv. Biochem. Psychopharmacol. 16, 645-648.

Cotes, P.M., Crow, T.J., Johnstone, E.C., Bartlett, W. and Bourne, R.C. (1978) Neuroendocrine changes in acute schizophrenia as a function of clinical state and neuroleptic medication. Psychol. Med. 8, 657-665.

Creese, I., Burt, D.R. and Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192, 481-483.

Cross, A.J., Crow, T.J. and Owen, F. (1981) ^{3}H-flupenthixol binding in post-mortem brains of schizophrenics: evidence for selective increase in dopamine D2 receptors. Psychopharmacol. 74, 122-124.

Crow, T.J., Johnstone, E.C. and McClelland, H.A. (1976) The coincidence of schizophrenia and Parkinsonism: some neurochemical implications. Psychol. Med. 6, 227-233.

Crow, T.J., Johnstone, E.C. and Owen, F. (1979) Research on schizophrenia. In: Recent Advances in Clinical Psychiatry (ed Granville-Grossman, K.) Volume 3. Churchill Livingstone, London.

Crow, T.J., Ferrier, I.N., Johnstone, E.C., McMillan, J.F.,

Owens, D.G.C., Parry, R.P. and Tyrrell, D.A.J. (1979) Characteristics of patients with schizophrenia or neurological disorder and virus-like agent in cerebrospinal fluid. Lancet 1, 842-844.

Crow, T.J. (1980) Molecular pathology of schizophrenia: more than one disease process? Brit. Med. J. 280, 66-8.

Crow, T.J., Owen, F., Cross, A.J., Ferrier, I.N., Johnstone, E.C., McCreadie, R.M., Owens, D.G.C. and Poulter, M. (1980) Neurotransmitter enzymes and receptors in post-mortem brain in schizophrenia: evidence that an increase in D2 dopamine receptors is associated with the Type I syndrome. In: Transmitter biochemistry of human brain tissue (ed Riederer, P. and Usdin, E.) 85-96.

Davison, K. and Bagley, C.R. (1969) Current problems in neuropsychiatry (ed Herrington, R.N.). Special publication No. 4 of Brit. J. Psychiat.

Deakin, J.F.W., Baker, H.F., Frith, C.D., Joseph, M.H. and Johnstone, E.C. (1979) Arousal related to excretion of noradrenaline metabolites and clinical aspects of unmedicated chronic schizophrenic patients. J. Psychiat. Res. 15, 57-65.

De Rivera, J.L., Lal, S., Ettigi, P., Mantella, S., Muller, H.F. and Freisen, H.G. (1976) Effect of acute and chronic neuroleptic therapy on serum prolactin levels in men and women of different age groups. Clin. Endocrinol. 5, 273-282.

Di Chiara, G., Corsini, G.U., Mereu, G.P., Tissari, A. and

Gessa, G.L. (1978) Self-inhibitory dopamine receptors: their role in the biochemical and behavioural effects of low doses of apomorphine. Adv. Biochem. Psychopharm. 19, 275-292.

Doerr, P., Fichter, M., Pirke, K.M. and Lund, R. (1981) On the regulation of cortisol secretion in anorexia nervosa. In: Biological Psychiatry (eds Perris, C., Struwe G. and Jansson, B.) North Holland: Elsevier 1047-1051.

Dom, R. (1976) Neostriatal and thalamic interneurones; their role in the pathophysiology of Huntington's Chorea, Parkinson's disease and catatonic schizophrenia. Thesis, University of Leuven.

Eberhart, J.A. and Keverne, E.B. (1979) Influences of the dominance hierarchy on luteinizing hormone, testosterone and prolactin in male talapoin monkeys. J. Endocrinol. 83, 42-43.

Emment, Y., Collins, W.P. and Somerville, I.F. (1972) Radio-immunoassay of oestrone and oestradiol in human plasma. Acta. Endocrinol. 69, 567-582.

Endo, M., Daiguji, M., Asano, Y., Yamashita, I. and Takahashi, S. (1978) Periodic psychosis recurring in association with the menstrual cycle. J. Clin. Psychiat. 39, 456-466.

Ettigi, P., Lal, S., Martin, J.B. and Friesen, H.G. (1975) Effect of sex, oral contraceptives and glucose loading on apomorphine-induced growth hormone secretion. J. Clin. Endocrinol. Metab. 40, 1094-1098.

Ettigi, P.G., Nair, N.P.W., Lal, S., Cervantes, P. and Guyda, H.

(1976) Effect of apomorphine on growth hormone and prolactin secretion in schizophrenic patients with or without oral dyskinesia, withdrawn from chronic neuroleptic therapy. J. Neurol. Neurosurg. Psychiat. 39, 870-876.

Feighner, J.P., Robins, E., Guze, S.B., Woodruff, R.A., Winokur, G. and Miroz, R. (1972) Diagnostic criteria for use in psychiatric research. Arch. Gen. Psychiat. 26, 57-63.

Feldman, F., Susselman, S. and Barrera, S.E. (1945) A note on apomorphine as a sedative. Am. J. Psychiat. 102, 403-405.

Ferrari, C., Rampini, P., Benco, R., Caldora, R., Scarduelli, C. and Crosiguani, P.G. (1982) Functional characterization of hypothalamic hyperprolactinaemia. J. Clin. Endocrinol. Metab. 55, 897-901.

Friend, W.C., Brown, G.M., Jawahir, G., Lee, T. and Seeman, P. (1978) Effect of haloperidol and apomorphine treatment on dopamine receptors in pituitary and striatum. Am. J. Psychiat. 135, 839-841.

Fuxe, K., Hökfelt, T. and Nilsson, O. (1969) Factors involved in the control of the activity of tubero-infundibular dopamine neurons during pregnancy and lactation. Neuro-endocrinol. 5, 257-270.

Gallo, R.V. (1980) Neuroendocrine regulation of pulsatile luteinizing hormone release in the rat. Neuroendocrinol. 30, 122-131.

Gerner, R.H. and Gwertsman, H.E. (1981) Abnormalities of dexamethasone suppression test and urinary MHPG in anorexia nervosa. Am. J. Psychiat. 138, 650-653.

Gil-Ad, I., Dicherman, Z., Weizman, R., Weizman, A., Tyamo, S. and Laron, Z. (1981) Abnormal growth hormone response to LRH and TRH in adolescent schizophrenic boys. Am. J. Psychiat. 138, 357-359.

Gimes, R., Toth, F. and Fornadi, F. (1969) Colpocytology, a prognostic aid in the treatment of schizophrenia. Acta. Cytol. 13, 133-135.

Glass, M.R., Shaw, R.W., Butt, W.R., Langan Edwards, R. and Landon, D.R. (1975) An abnormality of oestrogen feedback in amenorrhoea galactorrhoea. Brit. Med. J. 3, 274-275.

Glazer, W.M., Moore, D.C., Bowers, M.B. and Brown, W.A. (1981) Serum prolactin and tardive dyskinesia. Am. J. Psychiat. 138, 1493-1496.

Grossman, A., Moult, P.J.A., Gaillard, R.C., Delitala, G., Toff, W.D., Rees, L.H. and Besser, G.M. (1981) The opioid control of LH and FSH release: effects of a met-enkephalin analogue and naloxone. Clin. Endocrinol. 14, 41-47.

Gruen, P.H., Sacher, E.J., Altman, N., Leifer, M., Frantz, A.G. and Halpern, F.S. (1978) Prolactin responses to neuroleptics in normal and schizophrenic subjects. Arch. Gen. Psychiat. 35, 108-116.

Gudelsky, G.A., Simpkins, J., Mueller, G.P., Meites, J. and Moore, K.E. (1976) Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinol 22, 206-215.

Guillemin, R., Burgus, R. and Vale, W. (1971) The hypothalamic hypophysiotrophic thyrotrophin releasing factor. Vitam

Horm. 29, 1-13.

Halbreich, U., Sachar, E.J., Nathan, R.S., Asnis, G.M. and Halpern, F.S. (1980) The effect of benztropine mesylate on the prolactin response to haloperidol. Psychopharmacol. 72, 61-65.

Harris, G.W. and Jacobsohn, D. (1952) Functional grafts of anterior pituitary gland. Proc. Roy. Soc. Ser. B. 139, 263-270.

Healey, D.L. and Burger, H.G. (1977) Increased prolactin and thyrotrophin secretion following oral metoclopramide: dose response relationships. Clin. Endocrinol. 7, 195-201.

Healey, M.J.R. (1972) Statistical analysis of radioimmunoassay data. Biochem. J. 130, 207-210.

Hemphill, R.E., Reiss, M. and Taylor, A.L. (1944) Study of histology of testis in schizophrenia and other mental disorders. J. Ment. Sci. 90, 681-695.

Hemphill, R.E. and Reiss, M. (1945) Serum gonadotrophin and testes biopsy in the treatment of schizophrenia. J. Ment. Sci. 91, 1-7.

Hogarty, G.E., Goldberg, S.C., Schooler, N.R., Ulrich, R.F. and The Collaborative Study Group (1974) Drugs and sociotherapy in the aftercare of schizophrenic patients. II. Two year relapse rates. Arch. Gen. Psychiat. 31, 603-608.

Holden, M.M.C., Forno, G., Itil, T. and Hsu, W. (1973) Echo-encephalographic patterns in chronic schizophrenia (relationship to therapy resistance). Biol. Psych. 6, 129-141.

Hoskins, R.G. (1943) Psychosexuality in schizophrenia - some endocrine considerations. Psychosom. Med. 5, 3-9.

Hoskins, R.G. and Pincus, G. (1949) Sex-hormone relationships in schizophrenic men. Psychosom. Med. 11, 102-109.

Hunter, W.M. (1982) Labelling of protein antigens. In: Immunoassay for clinical chemistry (ed. Hunter, W.M. and Corrie, J.E.T.) Churchill Livingstone, Edinburgh. 7, 1-40.

Huseman, C.A., Kugler, J.A. and Schneider, I.G. (1980) Mechanism of dopaminergic suppression of gonadotrophin secretion in men. J. Clin. Endocrin. Metab. 51, 209-213.

Huws, D. and Groom, G.V. (1977) Luteinizing hormone-releasing hormone and thyrotrophin-releasing hormone stimulation studies in patients given clomipramine or 'depot' neuroleptics. Postgrad. Med. J. 53 (Suppl. 4), 175-181.

Hyyppa, M.T. (1978) Plasma pituitary hormones in patients with Parkinson's disease treated with bromocriptine. J. Neural. Trans. 42, 151-157.

Jacobson, R.I., Seyler, L.E., Tanborlane, W.V., Gertner, J.M. and Genel, M. (1980) Pulsatile subcutaneous nocturnal administration of GnRH by portable infusion pump in hypogonadotrophin hypogonadism: initiation of gonadotrophin responsiveness. J. Clin. Endocrinol. Metab. 49, 652-54.

Jeste, D.V. (1981) Apomorphine response in schizophrenia. Scientific Proceedings, American Psychiatric Association.

Johnstone, E.C., Crow, T.J., Frith, C.D., Husband, J. and Kreel, L. (1976) Cerebral ventricular size and cognitive impairment in chronic schizophrenia. Lancet ii, 924-926.

Johnstone, E.C., Crow, T.J. and Meshiter, K. (1977) Anterior pituitary hormone secretion in chronic schizophrenia - an approach to neurohumeral mechanisms. Psychol. Med. 7, 223-228.

Johnstone, E.C., Crow, T.J., Frith, C.D., Carney, M.W.P. and Price, J.S. (1978) Mechanism of the antipsychotic effect in acute schizophrenia. Lancet 1, 848-851.

Johnstone, E.C., Crow, T.J., Stevens, M., Kreel, L. and Husband, J. (1978) Dementia of dementia praecox. Acta Psychiat. Scand. 57, 305-324.

Johnstone, E.C. and Ferrier, I.N. (1980) Neuroendocrine markers of CNS drug effects. Brit. J. Clin. Pharmac. 10, 5-21.

Johnstone, E.C., Owens, D.G.C., Gold, A., Crow, T.J. and McMillan, J.F. (1981) Institutionalization and the defects of schizophrenia. Brit. J. Psychiat. 139, 195-203.

Kaptein, E.M., Kletzky, O.A., Spencer, C.A. and Nicoloff, J.T. (1980) Effects of prolonged dopamine infusion on anterior pituitary function in normal men. J. Clin. Endocrin. Metab. 51, 488-491.

Karson, C.N., Freed, W.J., Kleinman, J.E., Bigelow, L.D. and Wyatt, R.J. (1981) Neuroleptics decrease blink rates in schizophrenic patients. Biol. Psychiat. 7, 679-82.

Karson, G.N., Bigelow, L.B., Kleinman, J.E., Weinberger, D.R. and Wyatt, R.J. (1982) Haloperidol-induced changes in blink rates correlate with changes in BPRS score. Brit. J. Psychiat. 140, 503-507.

Kastin, A.J., Gual, C. and Schally, A.V. (1972) Clinical experience with hypothalamic releasing hormones. Part 2 Luteinizing hormone-releasing hormone and other hypophysiotrophic releasing hormones. Recent Prog. Hormone Res. 28, 201-206.

Kauppila, A. and Ylikorkala, O. (1982) Effect of oral and intravenous TRH and metoclopramide on PRL and TSH secretion in women. Clin. Endocrinol. 17, 617-623.

Kebabian, J.W. and Calne, D.B. (1979) Multiple receptors for dopamine. Nature 277, 93-96.

Kendall, R.E., Brockington, I.F. and Leff, J.P. (1979) Prognostic implications of six alternative definitions of schizophrenia. Arch. Gen. Psychiat. 36, 25-31.

Kleinman, J.E., Weinberger, D.R., Rogol, A.D., Bigelow, L.E., Klein, S.J., Gillin, C. and Wyatt, R.J. (1982) Plasma prolactin concentrations and psychopathology in chronic schizophrenia. Arch. Gen. Psychiat. 39, 655-657.

Kline, N.S., Blair, J., Cooper, T.B., Esser, A.H., Hackett, E. and Vestergaard, P. (1968) A controlled seven-year study of endocrine and other indices in drug treated chronic schizophrenics. Acta Psychiat. Scand. (Suppl.) 206.

Kolakowska, T., Wiles, D.H., McNeilly, A.S. and Gelder, M.G. (1975) Correlation between plasma levels of prolactin and chlorpromazine in pschiatric patients. Psychol. Med. 5, 214-216.

Kolakowska, T., Orr, M., Gelder, M., Heggie, M., Wiles, D. and Franklin, M. (1979) Clinical significance of plasma, drug and prolactin levels during acute chlorpromazine

treatment: a replication study. Brit. J. Psychiat. 135, 352-359.

Kornetsky, C. (1976) Hyporesponsivity of chronic schizophrenic patients to dexamphetamine. Arch. Gen. Psychiat. 33, 1425-1428.

Kraepelin, E. (1919) Dementia praecox (translated Barclay, R.M.) Facsimile edition. Kreiger, New York (1971).

Krawiecka, M., Goldberg, D. and Vaughan, M. (1977) A standardized psychiatric assessment for rating chronic psychotic patients. Acta Psychiat. Scand. 55, 299-308.

Krulich, L. (1982) Neurotransmitter control of thyrotrophin secretion. Neuroendocrinol. 35, 139-147.

Lachelin, G.C.L. and Yen, S.S.C. (1978) Hypothalamic chronic anovulation. Am. J. Obstet. Gynecol. 130, 825.

Lal, H., Brown, W., Drawburgh, R., Hynes, M. and Brown, G. (1977) Enhanced prolactin inhibition following chronic treatment with haloperidol and morphine. Life Sci. 20, 101-106.

Lal, S., De La Vega, C.E., Sourkes, T.L. and Friesen, H.G. (1973) Effect of apomorphine on growth hormone, prolactin, luteinizing hormone and follicle stimulating hormone in human serum. J. Clin. Endocrinol. Metab. 37, 719-724.

Lal, S., Martin, J.B., De La Vega, C. and Friesen, H. (1975) Comparison of the effect of apomorphine and L-dopa on the serum hormone levels in normal men. Clin. Endocrinol. 4, 277-285.

Lal, S., Harvey, G. and Bikadoroff, S. (1977) Effect of methysergide and pimozide on apomorphine induced growth

hormone secretion in man. J. Clin. Endocrinol. Metab. 44, 766-770.

Lal, S., Mendis, T., Cervantes, P., Guyda, H. and de Rivera, J.L. (1979) Effect of benztropine on haloperidol induced prolactin secretion. Neuropsychobiol. 5(6), 327-331.

Langer, G., Heinze, G., Reim, B. and Matussek, N. (1976) Reduced growth hormone responses to amphetamine in 'endogenous' depressive patients. Arch. Gen. Psychiat. 33, 1471-1475.

Langer, G., Sachar, E.J., Gruen, P.H. and Halpern, F.S. (1977) Human prolactin responses to neuroleptic drugs correlate with antischizophrenic potency. Nature 266, 639-640.

Langer, G., Sachar, E.J., Nathan, R.S., Tabrizi, M.A., Perez, J.M. and Halpern, F.S. (1979) Dopaminergic factors in human prolactin regulation, a pituitary model for the study of a neuroendocrine system in man. Psychopharmacol. 65, 161-164.

Lankford, H.V., Blackard, W.G., Gardner, D.F. and Tuder, H. St G. (1981) Effects of thyrotrophin-releasing hormone and metoclopramide in patients with phenothiazine-induced hyperprolactinaemia. J. Clin. Endocrinol. Metab. 53, 109-112.

Leblanc, H., Lachelin, G.C.L., Abu-Fadel, S. and Yen, S.S.C. (1976) Effect of dopamine infusion on pituitary hormone secretion in human. J. Clin. Endocrinol. Metab. 43, 668-674.

Lee, T., Seeman, P., Tourtellotte, W.W., Farley, I.J. and

Hornykeiwicz, O. (1978) Binding of ^{3}H-neuroleptics and ^{3}H-apomorphine in schizophrenic brains. Nature 274, 897-900.

Letemendia, F.J.J. and Harris, A.D. (1967) Chlorpromazine and the untreated chronic schizophrenic : a long term trial. Brit. J. Psychiat. 113, 950.

Lewis, N.O.C. and Davies, G.R. (1921) A correlative study of endocrine inbalances. J. Nerv. Ment. Dis. 54, 385.

List, S. and Seeman, P. (1981) Resolution of the dopamine and serotonin receptor components of ^{3}H-spiperone binding to rat brain regions. Proc. Nat. Acad. Sci. 78, 2620-2624.

Lucke, C. and Glick, S. (1971) Experimental modification of the sleep-induced peak of growth hormone secretion. J. Clin. Endocrinol. Metab. 32, 729-736.

Maeda, K., Kato, Y., Ohgo, S., Chihara, K., Yoshimato, Y., Yamaguchi, N., Kuromaru, S. and Imara, H, (1975) Growth hormone and prolactin release after injection of thyrotrophin-releasing hormone in patients with depression. J. Clin. Endocrinol. Metab. 40, 501-505.

Maeda, K., Kato, Y., Yamaguchi, N., Chichara, K., Ohgo, S., Iwasaki, Y., Yoshimoto, Y., Moridera, K., Kuromaru, S. and Imura, H. (1976) Growth hormone release following thyrotrophin-releasing hormone injection into patients with anorexia nervosa. Acta Endocrinol. 81, 1-8.

Marcano de Cotte, D., Menezes, C.E.L., Bennett, G.W. and Edwardson, J.A. (1980) Dopamine stimulates the degradation

of gonadotrophin releasing hormone by rat synaptosomes. Nature 283, 487-489.

Marshall, J.C. and Kelch, R.P. (1979) Low dose pulsatile gonadotrophin-releasing hormone in anorexia nervosa. J. Clin. Endocrinol. Metab. 49, 712-718.

Martin, J.B., Reichlin, S. and Brown, G.M. (1977) In: Clinical Neuroendocrinology, contemporary neurology. Series G.A. Davis Co. Philadelphia.

Mason, J.W. (1968) The scope of psychoendocrine research. Psychosom. Med. 30, 565-575.

Meites, J., Lu, K.H, Wuttke, W., Welsch, C.W., Ragasawa, H. and Qudri, S.K. (1972) Recent studies on functions and control of prolactin secretion in rats. Recent Prog. Horm. Res. 28, 471-527.

Meltzer, H.Y., Sachar, E.J. and Frantz, A.G. (1974) Serum prolactin levels in unmedicated schizophrenic patients. Arch. Gen. Psychiat. 31, 564-69.

Meltzer, H.Y. and Fang, V.S. (1976) The effect of neuroleptics on serum prolactin in schizophrenic patients. Arch. Gen. Psychiat. 33, 279-284.

Meltzer, H.Y. (1980) Relevance of dopamine autoreceptors for psychiatry : preclinical and clinical studies. Schizophrenia Bull. 6, 456-475.

Meltzer, H.Y., Busch, D. and Fang, V.S. (1982) Neuroendocrine abnormalities in schizophrenia: prolactin, growth hormone and gonadotrophins. In: Neuroendocrinology and psychiatric disorder (eds Brown, G.M., Reichlin, S. and Koslow, S.).

Raven Press, New York.

Michael, R.P. and Gibbons, J.L. (1963) Interrelations between the endocrine system and neuropsychiatry. Int. Rev. Neurobiol. 5, 243-302.

Morse, M.E. (1923) Pathological anatomy of ductless glands in series of dementia praecox. J. Neural. Psychopath. 4, 1.

Mott, F.W. (1919) The reproductive organs in relation to mental disorders. Brit. Med. J. 1, 463-467.

Moult, P.J.A., Grossman, A., Evans, J.M., Rees, L.H. and Besser, G.M. (1981) The effects of naloxone on pulsatile gonadotrophin release in normal subjects. Clin. Endocrinol. 14, 321-324.

Muller, E.E., Nistico, G. and Scapagini, E. (1977) In: Neurotransmitters and anterior pituitary function. New York, Academic Press.

Muller, P. and Seeman, P. (1978) Dopaminergic supersensitivity after neuroleptics: time course and specificity. Psychopharmacol. 60, 1-11.

Muller, E.E., Salerno, F., Cocchi, D., Locatelli, V. and Panerai, A.E. (1979) Interactions between the thyrotrophin-releasing hormone-induced growth hormone rise and dopaminegic drugs: studies in pathologic conditions of the animal and man. Clin. Endocrinol. 11, 645-656.

McCann, S.M., Ojeda, S.R., Martinovic, J. and Vijayan, E. (1977) Role of catecholamines, particularly dopamine, in the control of gonadotrophin secretion. In: Advances in biochemical psychopharmacology. Vol. 16 (ed. Costa, E. and Gessa, G.L.)

Raven Press, New York 109-114.

McCartney, J.L. (1929) Dementia praecox as an endocrinopathy with clinical and autopsy reports. Endocrinology 13, 73-87.

McLeod, R.M. and Lehemeyer, J.E. (1974) Studies on the mechanism of dopamine mediate inhibition of prolacting secretion. Endocrinology 94, 1077-1085.

McNeill, T.H. and Sladek, J.R. (1978) Fluorescence-immunocytochemisty: simultaneous localization of catecholamines and gonadotrophin-releasing hormone. Science 200, 72-74.

National Institute of Mental Health Psychopharmacology Service Centre Collaborative Study Group (1964) Phenothiazine treatment in acute schizophrenia. Arch. Gen. Psychiat. 246-261.

Noel, G.L., Suk, H.K., Stone, G.J., Frantz, A.G. (1972) Human prolactin and growth hormone release during surgery and other conditions of stress. J. Clin. Endocrinol. Metab. 35, 840-851.

Owen, F., Cross, A.J., Crow, T.J., Longden, A., Poulter, M. and Riley, G.J. (1978) Increased dopamine receptor sensitivity in schizophrenia. Lancet, ii, 223-226.

Owen, F., Cross, A.J., Crow, T.J., Lofthouse, R. and Poulter, M. (1981) Neurotransmitter receptors in brain in schizophrenia. Acta Psychiat. Scand. Suppl. 291, 63, 20-27.

Owen, F., Cross, A.J., and Crow, T.J. (1982) Neurotransmitter receptors in brain of schizophrenics in Cell Surface Receptors.(in press).

Owens, D.G.C. and Johnstone, E.C. (1980) The disabilities of chronic schizophrenia - their nature and factors contributing to their development. Brit. J. Psychiat. 136, 384-395.

Parkes, J.D., Debono, A.G. and Marsden, C.D. (1976) Growth hormone response in Parkinson's disease. Lancet i, 483.

Pandey, G.N., Garver, D.L., Tamminga, C., Erickson, S., Syad, I.A. and Davis, J.M. (1977) Post-synaptic supersensitivity in schizophrenia. Am. J. Psychiatry 134, 518-522.

Prange, A.J., Loosen, P.T., Wilson, I.C., Meltzer, H.Y. and Fang, V.S. (1979) Behavioural and endocrine responses of schizophrenic patients to TRH (Protirelin). Arch. Gen. Psychiat. 36, 1086-1093.

Quigley, M.E., Sheehan, K.L., Casper, R.F. and Yen, S.S.C. (1980) Evidence for increased dopaminergic and opioid activity in patients with hypothalamic hypogonadotrophic amenorrhoea. J. Clin. Endocrinol. Metab. 50, 949-953.

Randrup, A. and Munkvad, I. (1972) Evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. Orthomolec. Psychiat. 1, 2-7.

Rao, V.A. Rama, Bishop, M. and Coppen, A. (1980) Clinical state, plasma levels of haloperidol and prolactin: a correlation study in chronic schizophrenia. Brit. J. Psychiat. 137, 518-521.

Rey, J.H. and Coppen, A.J. (1959) Distribution of androgyny in mental patients. Brit. Med. J. ii, 1445-1447.

Ridley, R.M., Baker, H.F. and Scraggs, R.M. (1979) The time

course of the behavioural effects of amphetamine and their reversal by haloperidol in a primate species. Biol. Psychiat. 14, 753-765.

Ripley, H.S. and Papanicolaou, G.N. (1941) The menstrual cycle with vaginal smear studies in schizophrenia, depression and elation. Amer. J. Psychiat. 98, 567-570.

Rivera-Calimlim, L., Nasrallah, H., Strauss, J., Lasagna, L. (1976) Effect of dose, dosage schedules and drug interactions on plasma chlorpromazine levels. Am. J. Psychiat. 133, 646-652.

Roberts, E. (1977) The γ-aminobutyric acid system and schizophrenia. In: Neuroregulators and Psychiatric Disorders (eds Usdin, E., Hamburg, D.A. and Barchas, J.D.) 347-357. Oxford University Press.

Robinson, J.D. and Risby, D. (1977) Radioimmunoassay for flupenthixol in plasma. Clin. Chem. 23, 2085-2088.

Rose, R.M., Bourne, P.G., Poe, N.D., Mongey, E.H., Collins, D.R. and Mason, J.W. (1969) Androgen response to stress. Psychosom. Med. 31, 418-436.

Rosenzweig, S. and Freeman, H. (1942) A "blind test" of sex-hormone potency in schizophrenic patients. Psychosom. Med. 4, 159-165.

Roth, J.C., Kelch, R.P., Kaplan, S.L. and Grumbach, M.M. (1972) FSH and LH response to luteinizing hormone-releasing factor in prepubertal and pubertal children, adult males and patients with hypogonadotropic and hypergonadotropic hypogonadism. J. Clin. Endocrinol. Metab. 35, 926-30.

Rotrosen, J., Angrist, B.M., Gershon, S., Sachar, E.J. and Halpern, F.S. (1978) Suppression of prolactin by dopamine agonists in schizophrenics and controls. Am. J. J. Psychiat. 135, 949-951.

Rotrosen, J., Angrist, B., Gershon, S., Parquin, T., Brenchey, L., Oleshansky, M., Halpern, F. and Sachar, E.J. (1979) Neuroendocrine effects of apomorphine. Characterisation of response patterns and application to schizophrenia research. Brit. J. Psychiat. 135, 444-456.

Sands, D.E. (1957) Endocrine changes in schizophrenia In: Schizophrenia (ed. Richler, D.) London.

Santen, R.J. and Bardin, C.W. (1973) Episodic luteinizing hormone secretion in man:- pulse analysis, clinical interpretation, physiologic mechanisms. J. Clin. Invest. 52, 2617-2628.

Santen, R.J. (1975) Is aromatization of testosterone to estradiol required for the inhibition of luteinizing hormone in men. J. Clin. Invest. 56, 1555-1563.

Sassin, J.F., Frantz, A.G., Weitzman, E.D. and Kapen, S. (1972) Human prolactin: 24-hour pattern with increased release during sleep. Science 177, 1205-1207.

Schally, A.V., Kastim, A.J. and Arimura, A. (1973) Hypothalamic regulatory hormones. Science 179, 341-350.

Schneider, K. (1959) Klinische Psychopathologie, translation of 5th Edition by Hamilton, M.W. Grune & Stratton, New York.

Shader, R.I., Taymoor, M.I. and Grimspoon, L. (1968) Schizophrenia,

oligospermia and the phenothiazines II. Studies on follicle stimulating hormone. Proc. IV World Congress Psych. p 2988-2991.

Shader, R.I. and Di Mascio, A. (1970) Psychotropic drug side effects. Baltimore, Williams and Wilens.

Shields, J. (1976) Genetics in schizophrenia. In: Schizophrenia today (Kemali, D., Bartholini, G. and Richter, D. eds) Pergammon Press, Oxford.

Simpson, G.M., Cooper, T.B., Bark, N., Sud, I. and Lee, J.H. (1980) Effect of antiparkinsonian medication on plasma levels of chlorpromazine. Arch. Gen. Psychiat. 37, 205-208.

Slater, D. (1962) The schizophrenia-like psychoses of epilepsy. Proc. Roy. Soc. Med. 55, 315-316.

Spitz, I.M., Zylber, E., Cohen, H., Almaliach, U. and Deroith, D. (1979) Impaired prolactin response to thyrotrophin-releasing hormone in isolated gonadotrophin deficiency and exaggerated response in primary testicular failure. J. Clin. Endocrinol. Metab. 48, 941-945.

Spokes, E.G.S., Garrett, N.J., Rossor, M.N. and Iversen, L.L. (1980) Distribution of GABA in post-mortem brain tissue from control, psychotic and Huntington's chorea subjects. J. Neurol. Sci. 48, 303-313.

Stanley, M., Lauten, A., Rotrosen, J. and Gershon, S. (1979) Antipsychotic efficacy of metoclopramide: do DA/neuroleptic receptors mediate the action of antipsychotic drugs? IRCS Med. Sci. 7, 322.

Stein, L. and Wise, C.D. (1971) Possible etiology of schizophrenia: progressive damage to the noradrenergic reward system by 6-hydroxydopamine. Science 171, 1032-1036.

Stevens, B.C. (1969) Marriage and fertility of women suffering from schizophrenia or affective disorders. Maudsley Monographs 19, Oxford University Press, London.

Stevens, J.R. (1978) Blink rates: psychosis or tardive dyskinesia? Am. J. Psychiat. 135, 223-7.

Stevens, J.R. (1981) Neuropathologic findings in schizophrenia In: Biological Psychiatry (eds C. Perris, G. Struwe, B. Jansson), Elsevier/North Holland Biomedical Press.

Taché, Y., Du Ruisseau, P., Ducharme, J.R. and Collu, R. (1978) Pattern of adenohypophyseal hormone changes in male rats following chronic stress. Neuroendocrinol. 26, 208-219.

Takahashi, R., Inaba, Y., Inanga, K., Kato, N., Kumashiro, H., Nishimura, T., Okuma, T., Otsuki, S., Saki, T., Sato, T. and Shimazono, Y. (1981) CT scanning and the investigation of schizophrenia. In: Biological Psychiatry (eds. Perris, C., Struwe, G. and Jansson, B.) Elsevier/North Holland Biomedical Press.

Tamminga, C.A., Smith, R.C., Pandey, G., Frohman, L.A. and Davis, J.M. (1977) A neuroendocrine study of supersensitivity in tardive dyskinesia. Arch. Gen. Psychiat. 34, 1199-1203.

Tamminga, C.A., Schaffer, M.H., Smith, R.H. and Davis, J.M. (1978) Schizophrenic symptoms improve with apomorphine. Science 200, 567-568.

Tamminga, C.A. and Schaffer, M.H. (1979) Treatment of schizophrenia with ergot derivatives. Psychopharmacol. 66, 239-242.

Tanner, J.M. (1951) Current advances in the study of physique: photographic anthropometry and an andorgyny scale. Lancet 1, 574-579.

Torrey, E.F. and Peterson, M.R. (1974) Schizophrenia and the limbic system. Lancet ii, 942-6.

Touitou, Y., Fevre, M., Lagoguey, M., Carayon, A., Bogdam, A., Reinberg, A., Beck, H., Cesselin, F. and Touitou, C. (1981) Age- and mental health-related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle-stimulating hormone in man. J. Endocr. 91, 467-475.

Valk, T.W., Corley, K.P., Kelch, R.P. and Marshall, J.C. (1980) Hypogonadotrophic hypogonadism: hormonal responses to low dose pulsatile administration of gonadotrophin-releasing hormone. J. Clin. Endocrinol. 51, 730-38.

Weinberger, D.R., Torrey, E.F., Neurophytides, A.N. and Wyatt, R.J. (1979) Cerebral ventricular enlargement in chronic schizophrenia. Arch. Gen. Psychiat. 36, 735-739.

Weiner, R.I. and Ganong, W.F. (1978) Role of monoamines and histamine in the regulation of anterior pituitary hormone secretion. Physiol. Rev. 58, 905-976.

Wells, B., Silverstone, T. and Rees, L. (1978) The effect of oral dextroamphetamine on PRL secretion in man. Neuropharmacol. 17, 1060-1061.

Whalley, L.J. (1982) Diagnostic value of endocrine tests in psychiatry. In: Neuropeptides: basic and clinical aspects (eds. Fink, G.

and Whalley, L.J.) Churchill Livingstone, Edinburgh.

W.H.O. Collaborating Centre Manual (1980) Available from Dr. S. Sufi, WHO Collaborating Centre for research and reference services for immunoassay for hormones in human reproduction. Dovehouse Street, London, SW3.

Wide, L., Nillius, S.J., Gerrizell, C. and Roos, P. (1973) Radioimmunoassay of FSH and LH in serum and urine from men and women. Acta Endocrinol. (Suppl) 174, 3-58.

Wiles, D.H., Kolakowska, T., McNeilly, A.S., Mandelbrote, B.M. and Gelder, M.G. (1976) Clinical significance of plasma chlorpromazine levels. I Plasma levels of the drugs, some of its metabolites and prolactin during acute treatment. Psychol. Med. 6, 407-415.

Wing, J.K., Cooper, J.E. and Sartorius, N. (1974) Measurement and Classification of Psychiatric Symptoms. Cambridge University Press : London.

Wing, J.K. and Nixon, J. (1975) Discriminating symptoms in schizophrenia. Arch. Gen. Psychiat. 32, 853-859.

Withers, E. and Hinton, J. (1971) Three forms of the clinical tests of the sensorium and their reliability. Brit. J. Psychiat. 119, 1-8.

Woolley, D.W. and Shaw, E. (1954) A biochemical and pharmacological suggestion about certain mental disorders. Proc. Nat. Acad. Sci. 40, 228-231.

Wuttke, W., Hanche, J.L., Hohn, K.G. and Baumgarten, A. (1979) Effect of intraventricular injection of 5, 7 dihydroxy-tryptamine on serum gonadotrophins and prolactin. Ann. N. Y. Acad. Sci. 423-436.